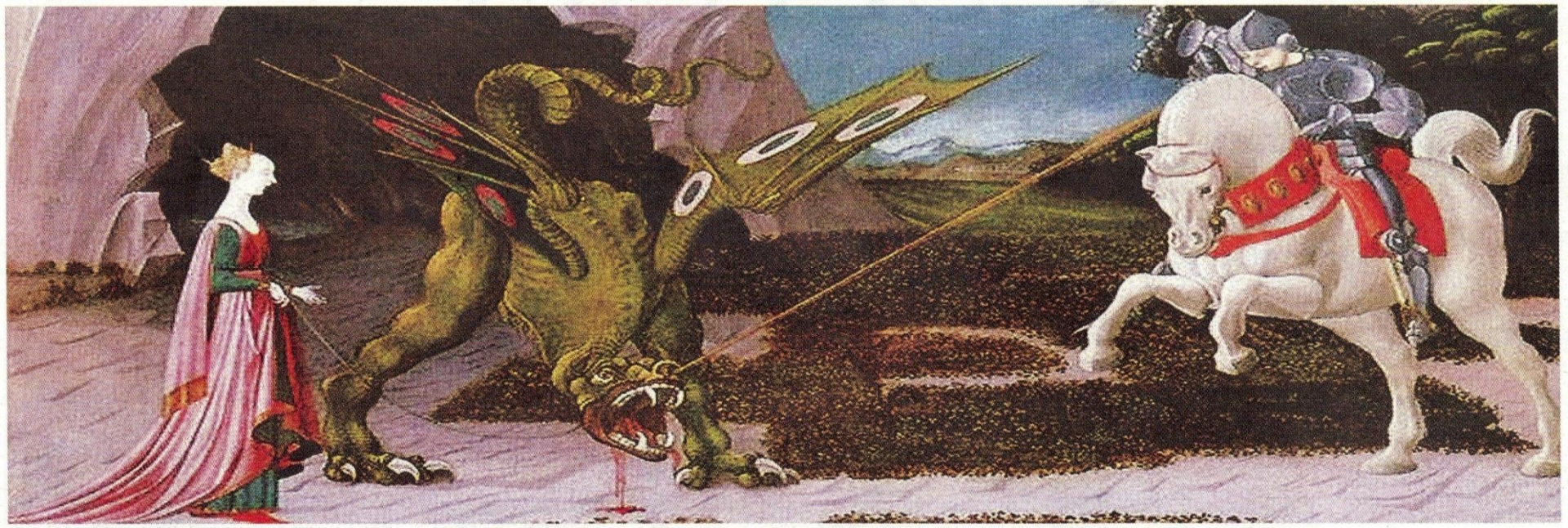
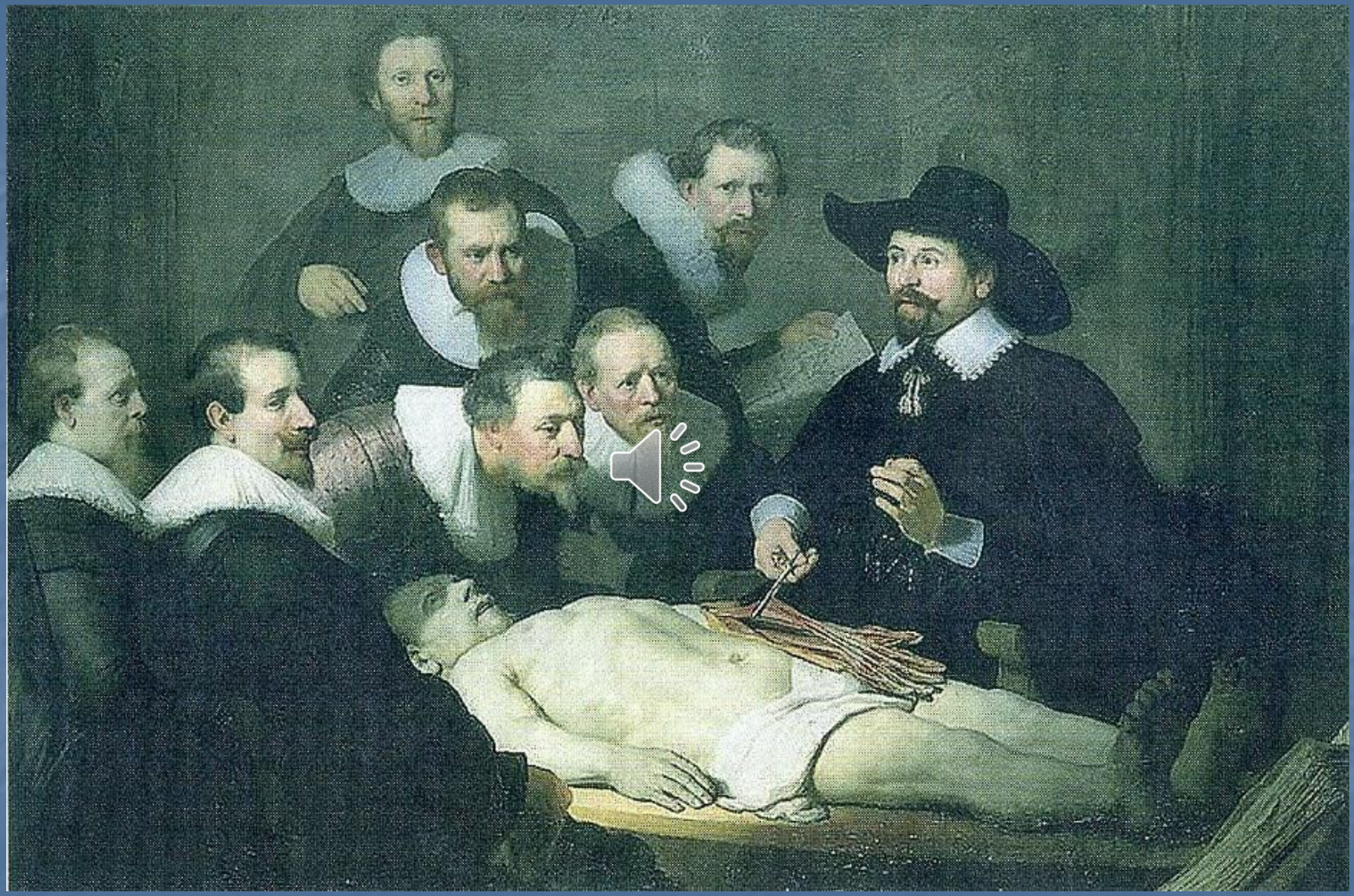
2nd International Conference on
Applied Microbiology and Beneficial Microbes
October 23-25, 2017 Osaka, Japan

Non-small cell lung (NSCL) cancer search for biomarkers from body fluids to microarrays



Prof. Giulio Tarro
Emeritus Chief "D. Cotugno" Hospital, Naples
Chairman of the Committee on Biotechnologies and VirusSphere, WABT - UNESCO, Paris
Rector of the University Thomas More U.P.T.M., Rome
President Foundation Teresa & Luigi de Beaumont Bonelli for Cancer Research



For Giulio Tarro – my scientific son – with great admiration and best wishes for a happy and successful life,

Albert B. Sabin June, 1968

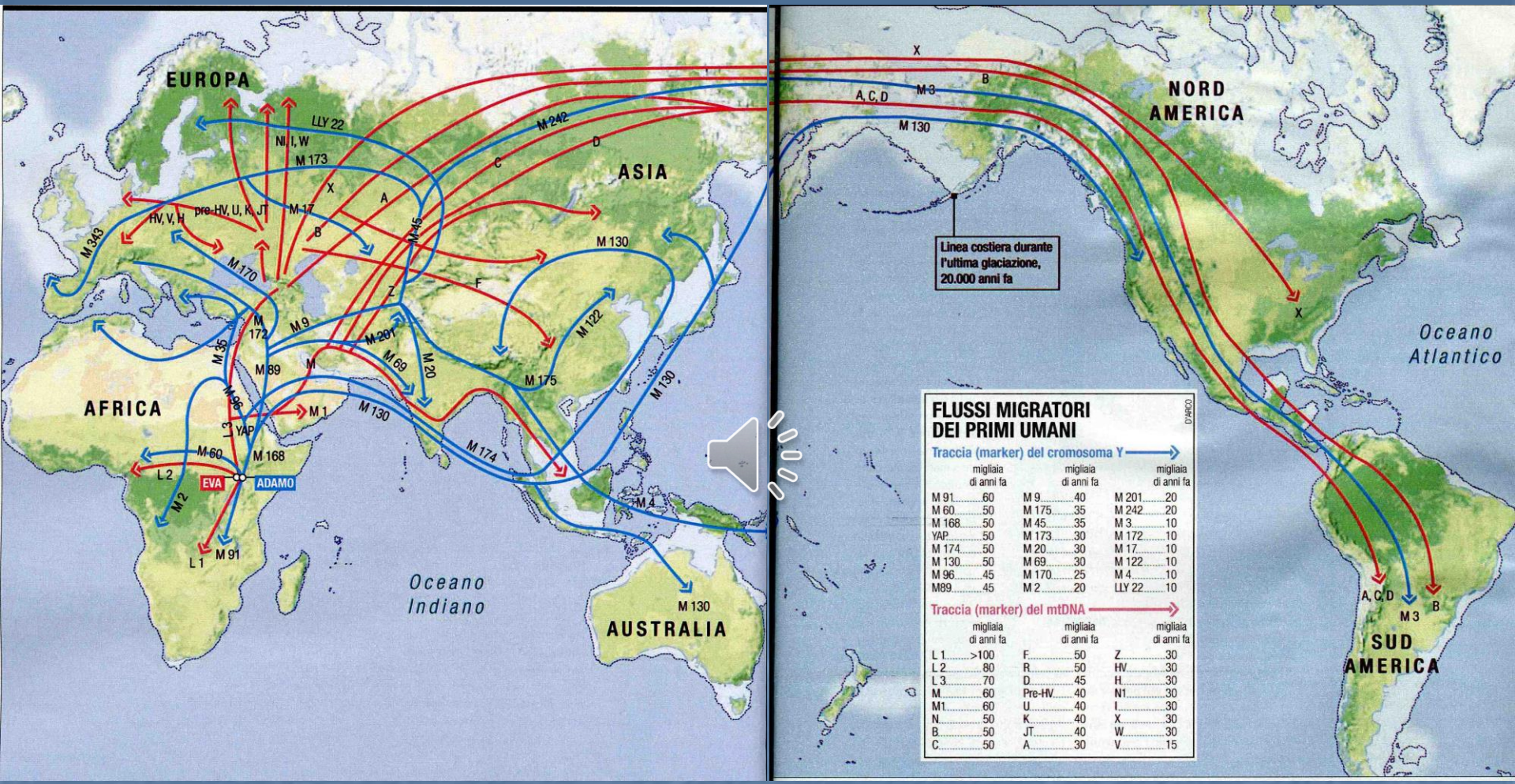
Theory Clin. Pract. Pediatr., 2017, 1(1), 2–3
DOI: 10.25082/TCPP.2017.01.002

COMMENTARY

Exegesis of Sabin poliovaccine in terms of medical science

Giullio Filippo Tarro


Virosphere 2002
Invertebrates
Vertebrates
Bacteria
Plants
Invertebrates
Vertebrates
Vertebrates Invertebrates
Plants
ssRNA+
Bacteria
Fungi
ssRNA-
Vertebrates
Invertebrates
Plants
dsRNA
Bacteria
Fungi
dsDNA-RT
ssRNA-RT
dsDNA
ssDNA
ssRNA-
ssRNA+
DONALD DANFORTH
PLANT SCIENCE CENTER
copyright©2002 C.M.Fauquet
International Committee on Taxonomy of Viruses


EUROPA
ASIA
AFRICA
NORD AMERICA
SUD AMERICA
AUSTRALIA
Oceano Atlantico
Oceano Indiano
LLY 22
NI, I, W
M 173
HV, V, H
pre-HV, U, K, JT
M 17
M 343
M 170
M 242
M 45
M 130
M 122
M 175
M 201
M 69
M 20
M 9
M 172
M 35
M 89
M 96
M 1
M 130
M 174
M 4
YAP
M 60
M 168
L 2
EVA
ADAMO
M 2
L 1
M 91
X
A
B
C
D
Z
F
A, C, D
M 3
B
Linea costiera durante l'ultima glaciazione, 20.000 anni fa
FLUSSI MIGRATORI DEI PRIMI UMANI
D'ARCO
Traccia (marker) del cromosoma Y
migliaia di anni fa
M 91 60
M 60 50
M 168 50
YAP 50
M 174 50
M 130 50
M 96 45
M89 45
M 9 40
M 175 35
M 45 35
M 173 30
M 20 30
M 69 30
M 170 25
M 2 20
M 201 20
M 242 20
M 3 10
M 172 10
M 17 10
M 122 10
M 4 10
LLY 22 10
Traccia (marker) del mtDNA
migliaia di anni fa
L 1 >100
L 2 80
L 3 70
M 60
M1 60
N 50
B 50
C 50
F 50
R 50
D 45
Pre-HV 40
U 40
K 40
JT 40
A 30
Z 30
HV 30
H 30
N1 30
I 30
X 30
W 30
V 15

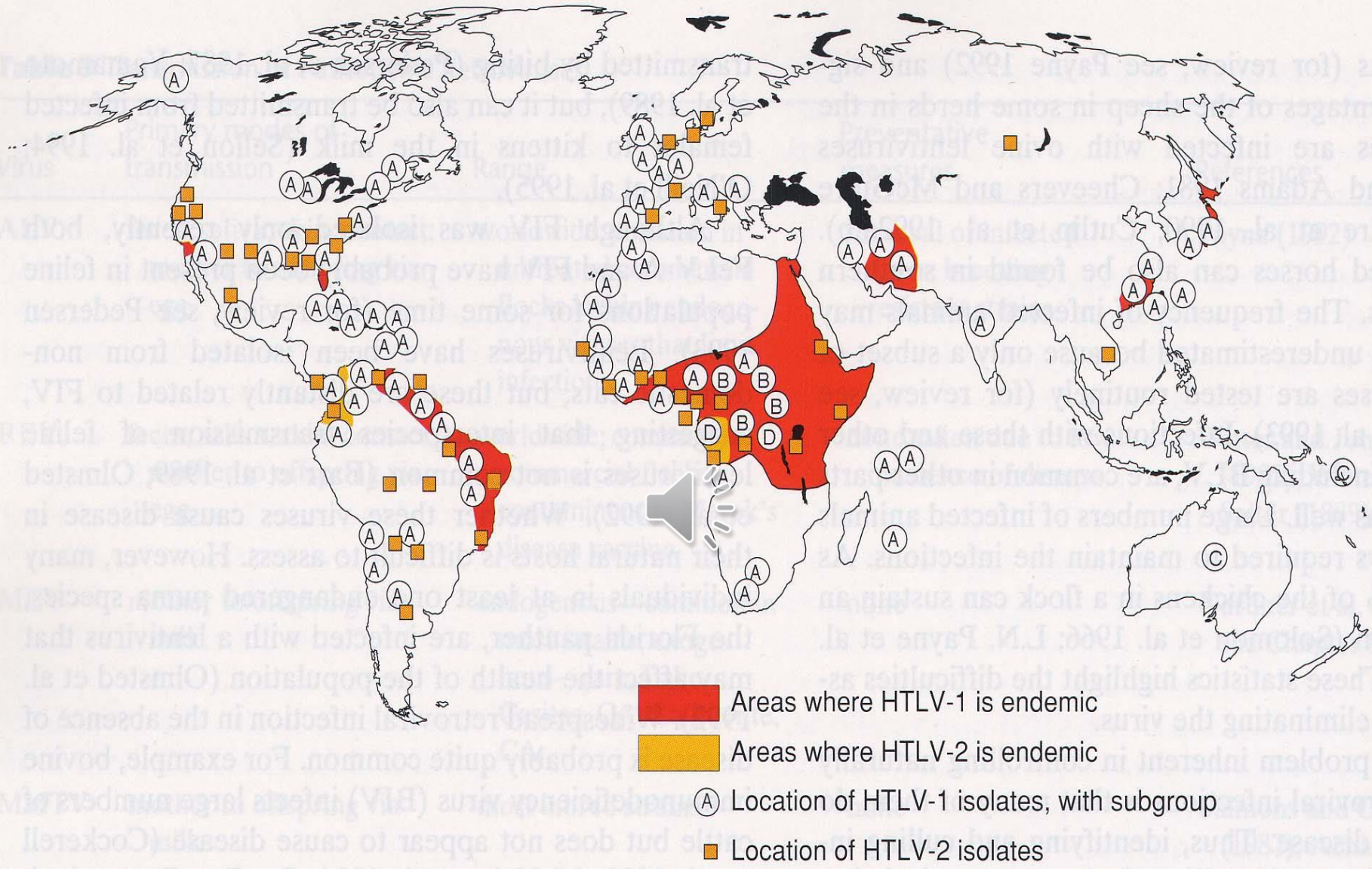
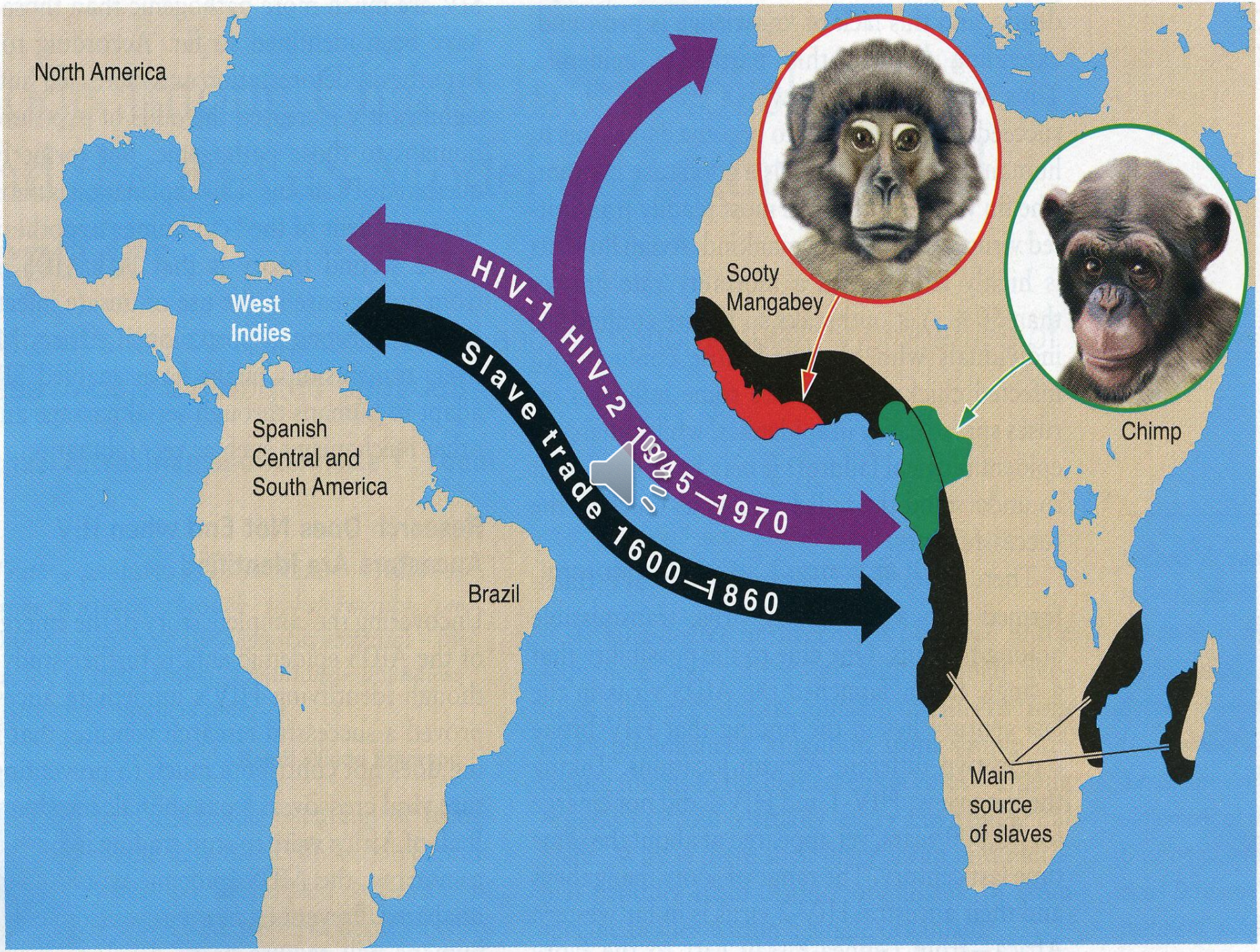
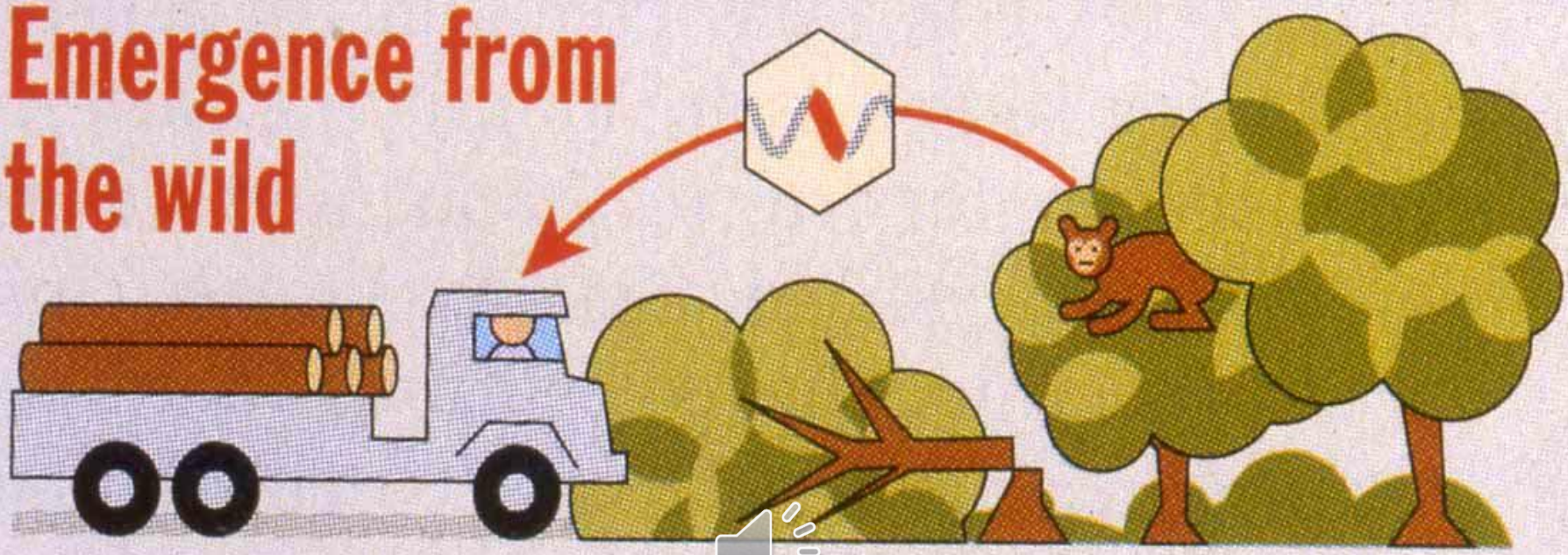


Figure 21 HTLV-1 and HTLV-2 distribution. The map illustrates the distribution of HTLV-infected individuals. (*A*) HTLV-1 cosmopolitan; (*B*) HTLV-1 Zaire (Central Africa); (*C*) HTLV-1 Melanesia; (*D*) HTLV-1 found in pygmies from Central Africa; (*gray boxes*) HTLV-2. (Modified from a figure provided by A. Gessain and G. Franchini.)

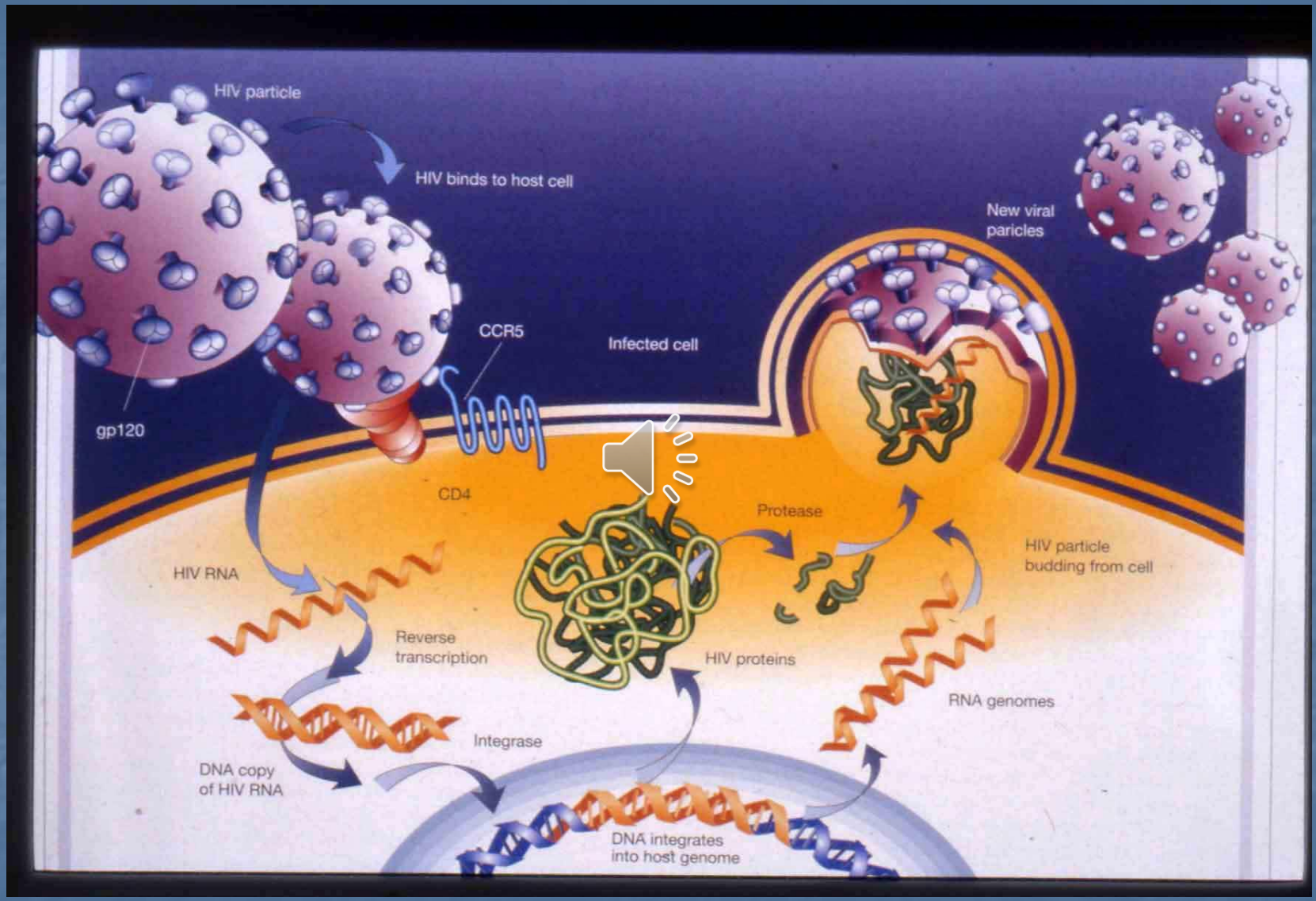

North America
West Indies
Spanish Central and South America
Brazil
HIV-1 HIV-2 1945—1970
Slave trade 1600—1860
Sooty Mangabey
Chimp
Main source of slaves

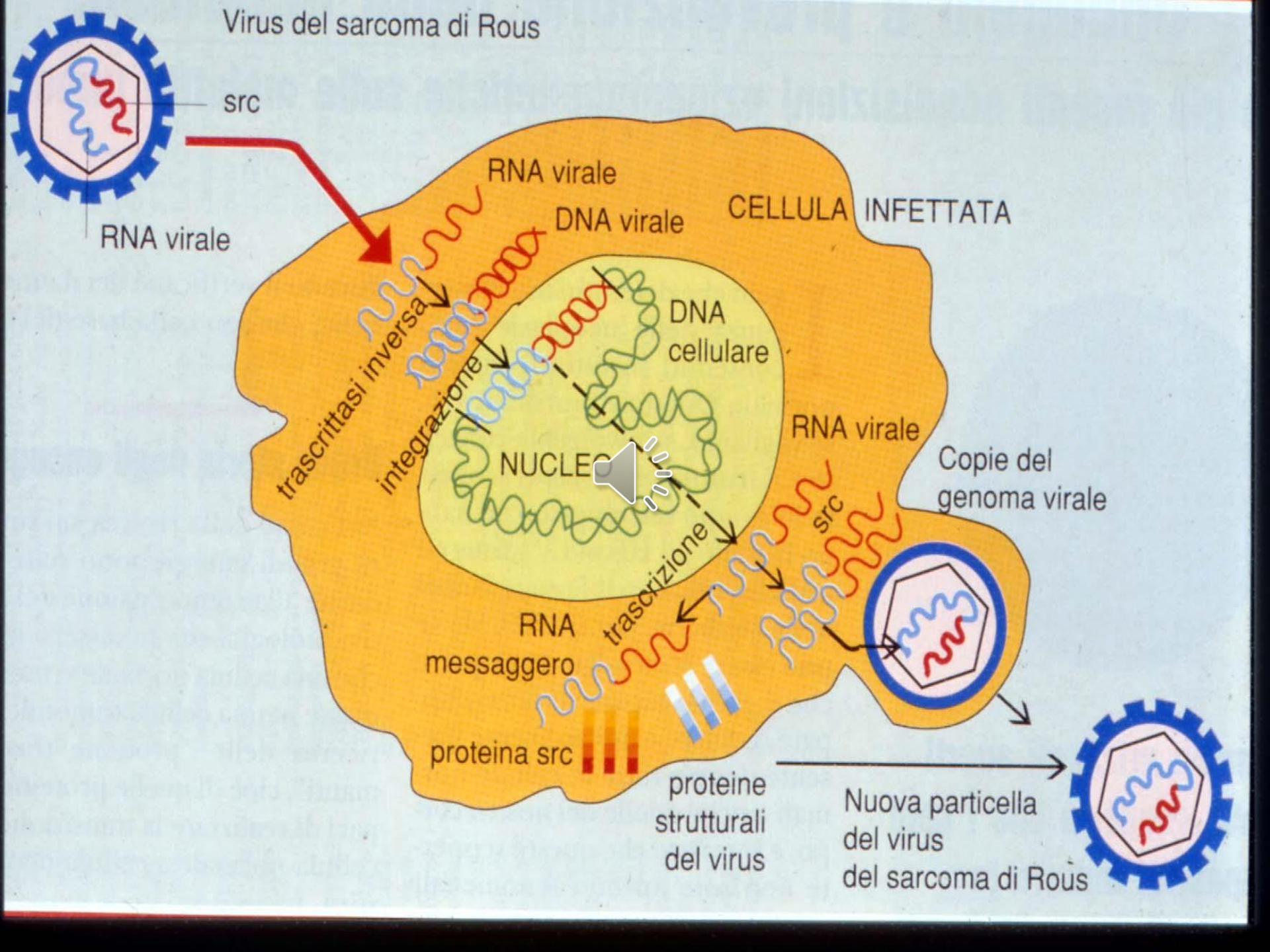
Emergence from the wild

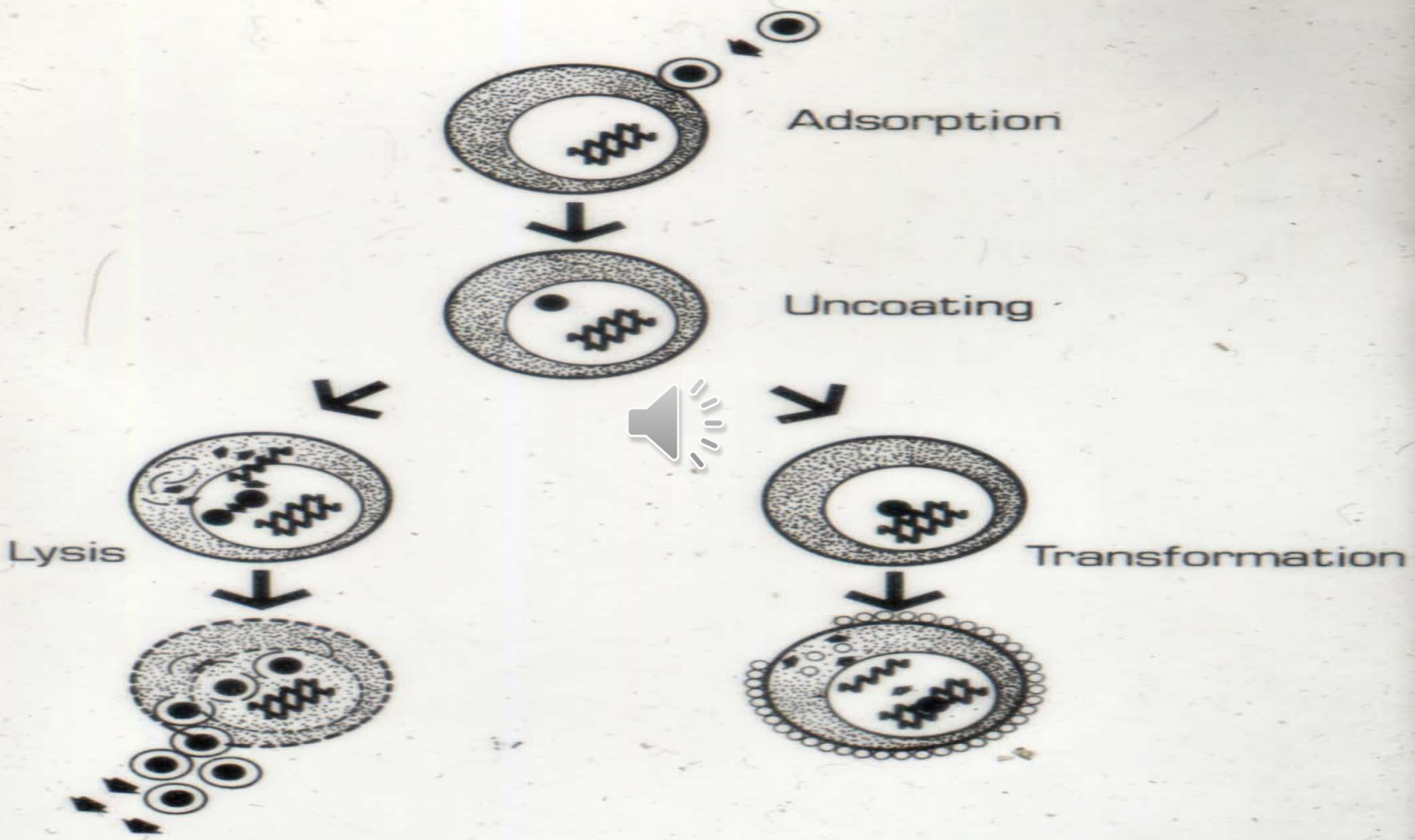


Some viruses lie hidden in environments that are isolated from humans. For example, when a forest is cleared, people may come into contact with animals carrying such viruses. If humans are infected, they can carry the disease back to populated areas. The AIDS virus may have entered the human population this way.

TIME Graphic by Joe Lertol


HIV particle
HIV binds to host cell
New viral
paricles
CCR5
Infected cell
gp120
CD4
Protease
HIV particle
budding from cell
HIV RNA
Reverse
transcription
HIV proteins
RNA genomes
Integrase
DNA copy
of HIV RNA
DNA integrates
into host genome


Virus del sarcoma di Rous
src
RNA virale
RNA virale
DNA virale
CELLULA INFETTATA
trascrittasi inversa
integrazione
DNA cellulare
NUCLEO
RNA virale
Copie del genoma virale
src
trascrizione
RNA messaggero
proteina src
proteine strutturali del virus
Nuova particella del virus del sarcoma di Rous


Adsorption
Uncoating
Lysis
Transformation

I virus oncogeni

Cancer AssociatedViruses of Man

- **Proven**

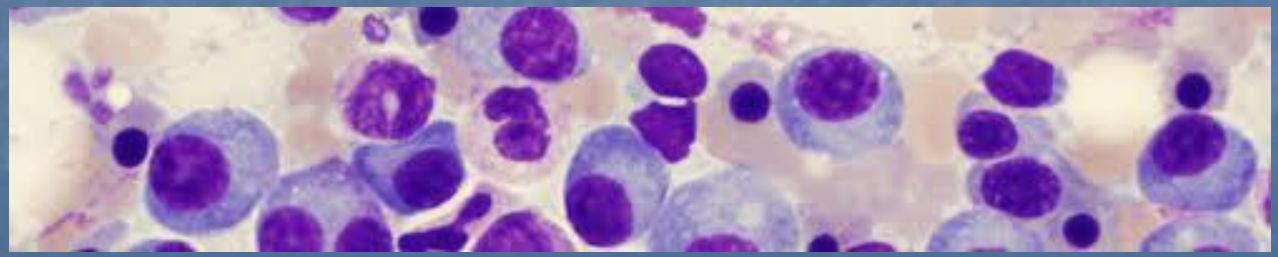
Certain strains of papillomavirus (Papovaviridae)
Epstein-Barr virus (Herpesviridae)
Hepatitis B virus (Hepadnaviridae)
HTLV-I and –II (Retroviridae)
Human herpes virus-8 (Herpesviridae)
Merkel cell polyomavirus (MCV)

- **Suspect**

Hepatitis C virus (Flaviviridae)
Herpes simplex virus (cofactor) (Herpesviridae)
HIV-1 and -2 (Retroviridae)
Polyomavirus (BKV, JCV) (Papovaviridae)

- **Possible**

Adenovirus (Adenoviridae)



Examples of Licensed and Experimental Vaccines against Established or Putative Virus Cancers of Man

- **Licensed**

Hepatitis B (plasma-derived and recombinant)

Adenovirus (live and killed)

Papillomavirus

- **Experimental-Investigative**

Retrovirus

HIV-1 and -2	**AIDS**
HTLV-1 and HTLV-2	**Leukemia**

Epstein-Barr virus

Hepatitis C

Source: Modified from annals N.Y. Academy of Science

ON THE COVER

Vaccines are our best defenses against many infectious diseases, having saved millions of lives and prevented immeasurable suffering. Yet today, in many developed countries, these defenses are fraying as parents, swayed by persistent untruths about the risks, hesitate to vaccinate their children. This special package sizes up the actual, rare risks of vaccines and what is known about how to overcome groundless fears. See page 364. *Illustration: Ben Wiseman*

FEATURES

THE VACCINE WARS

Debunking myths, owning real risks, and courting doubters

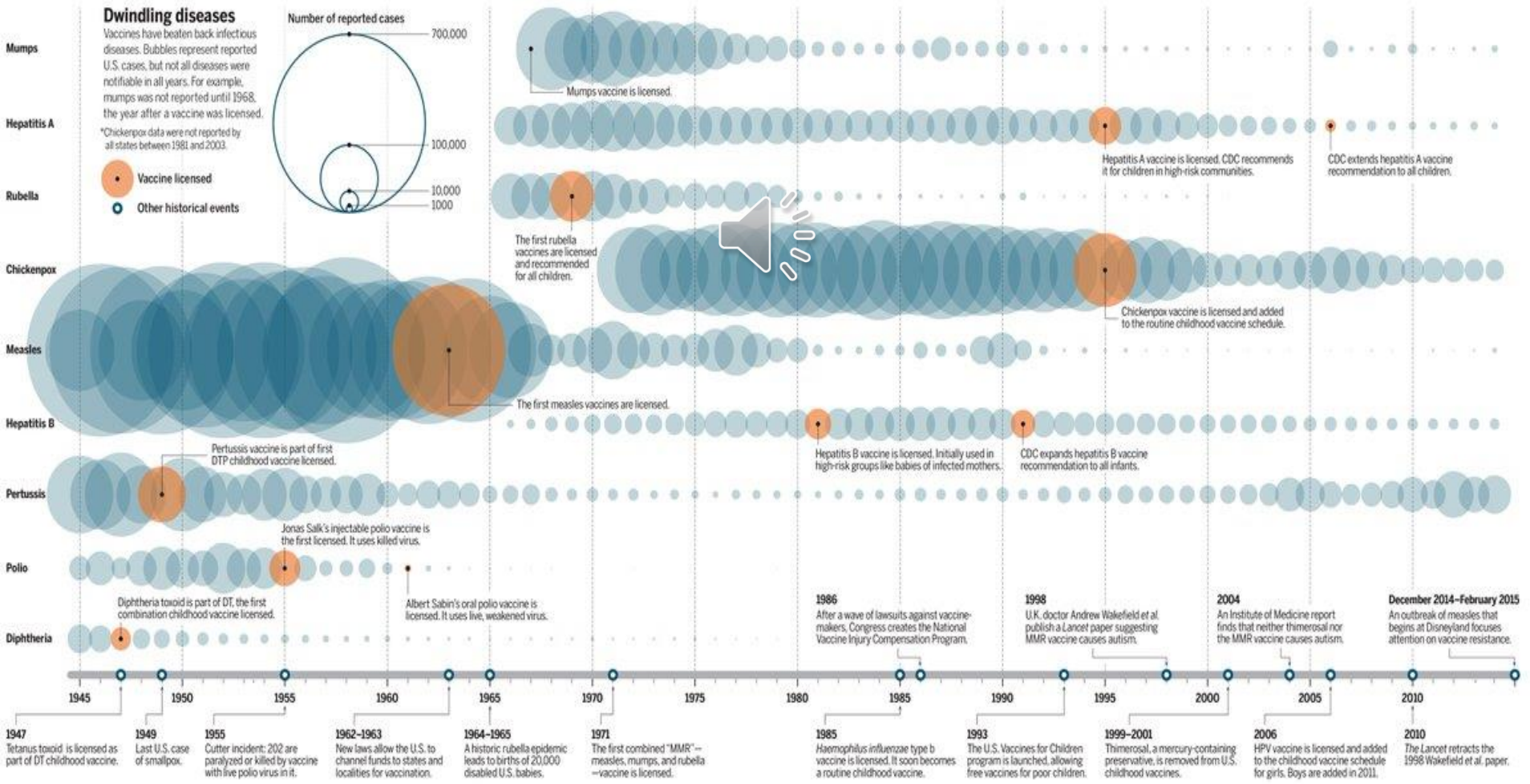
By **Meredith Wadman** *and* **Jia You**

LAST WEEK, PUBLIC HEALTH AUTHORITIES in Minnesota asked more than 200 people to quarantine themselves after 12 cases of measles were diagnosed in less than 2 weeks—all of them in unvaccinated children younger than 6 years. Across the ocean, an unvaccinated 17-year-old Portuguese girl died of measles after the virus invaded her lungs, in the midst of an outbreak there that mirrors surges in cases in Germany, Italy, and Romania.

In 2015, the most recent year for which data are available, just 72% of U.S. toddlers had received seven key vaccines recommended by the Centers for Disease Control and Prevention (CDC), which together protect against 11 potentially deadly diseases. That is actually an improvement from 2011, when the number was 69%; but it also indicates that much work remains to be done, particularly in an environment in which vaccine skeptics have been emboldened, not least by the current occupant of the Oval Office.

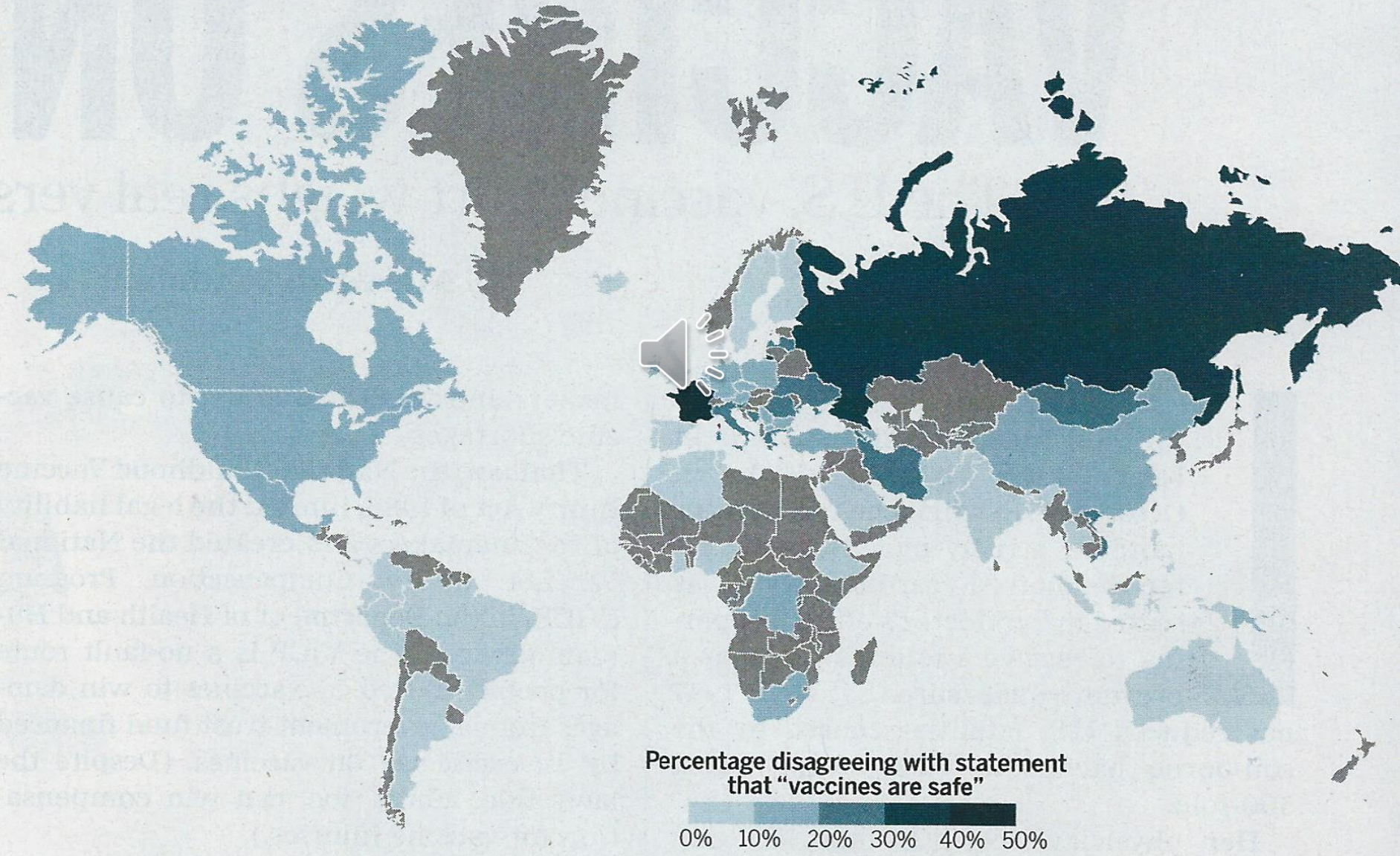
As once common diseases of childhood fade from public view, it is understandable that parents' attention would shift from the fear of disease to concerns about risks of the vaccines themselves. The articles in this issue debunk myths old and new about these risks, while acknowledging the real, rare vaccine injuries that do occur. The data on these pages make clear the power of vaccines to vanquish disease—an impact that far eclipses their minute risks. Identifying the best ways to convince hesitant parents of this calculus in an age of internet-fed misinformation is an ongoing challenge for researchers. ■

DATA: CDC



A matter of trust

A 2016 survey in 67 countries found that trust in vaccines is high overall but varies by country. Safety concerns were highest in Europe and Russia; in France, 41% disagreed with the statement that vaccines are safe.



FEATURES

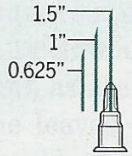
Shooting for success

Shoulder injuries are by far the commonest claims at the vaccine court. They result when a needle penetrates the shoulder joint, causing inflammation of tendons and fluid-filled bursas.

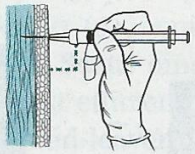
Do no harm

Patient and vaccinator both seated.
Lowers risk of aiming from above.

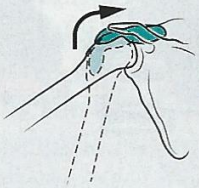
Aim for middle of triangle.
Avoiding upper third ensures delicate structures are not touched.



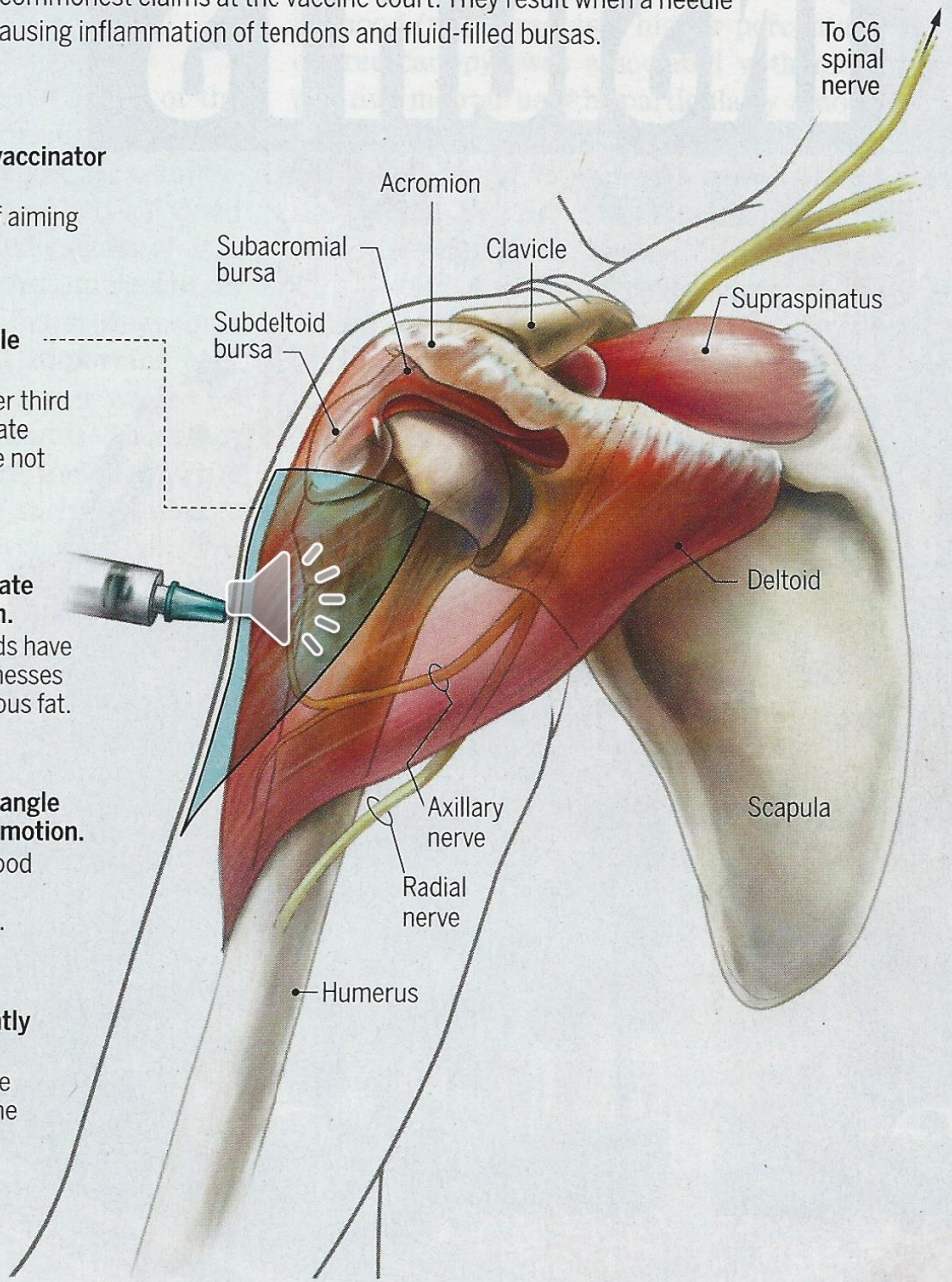
Use appropriate needle length.
Different builds have various thicknesses of subcutaneous fat.



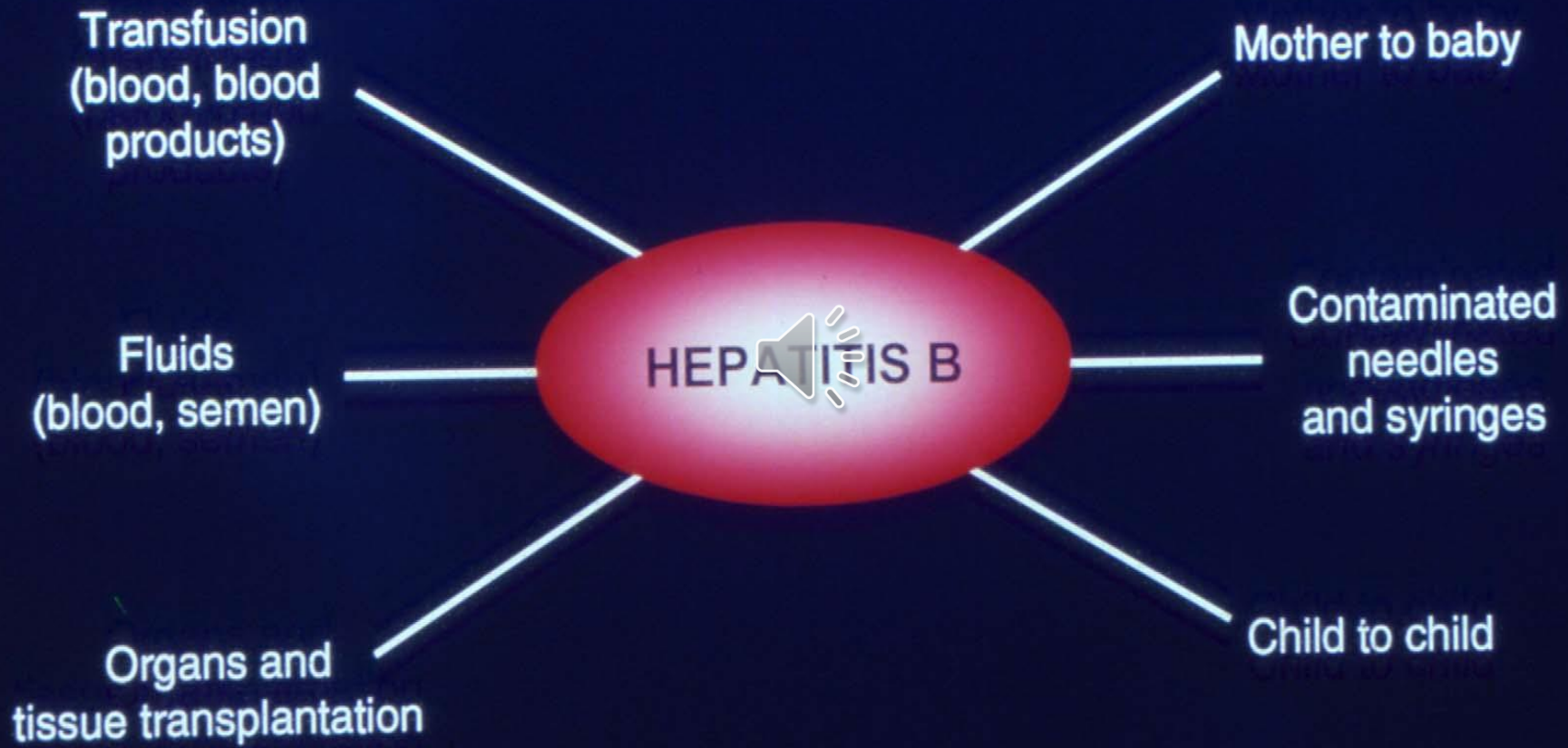
Insert at 90° angle with dartlike motion.
Higher likelihood of reaching muscle depth.



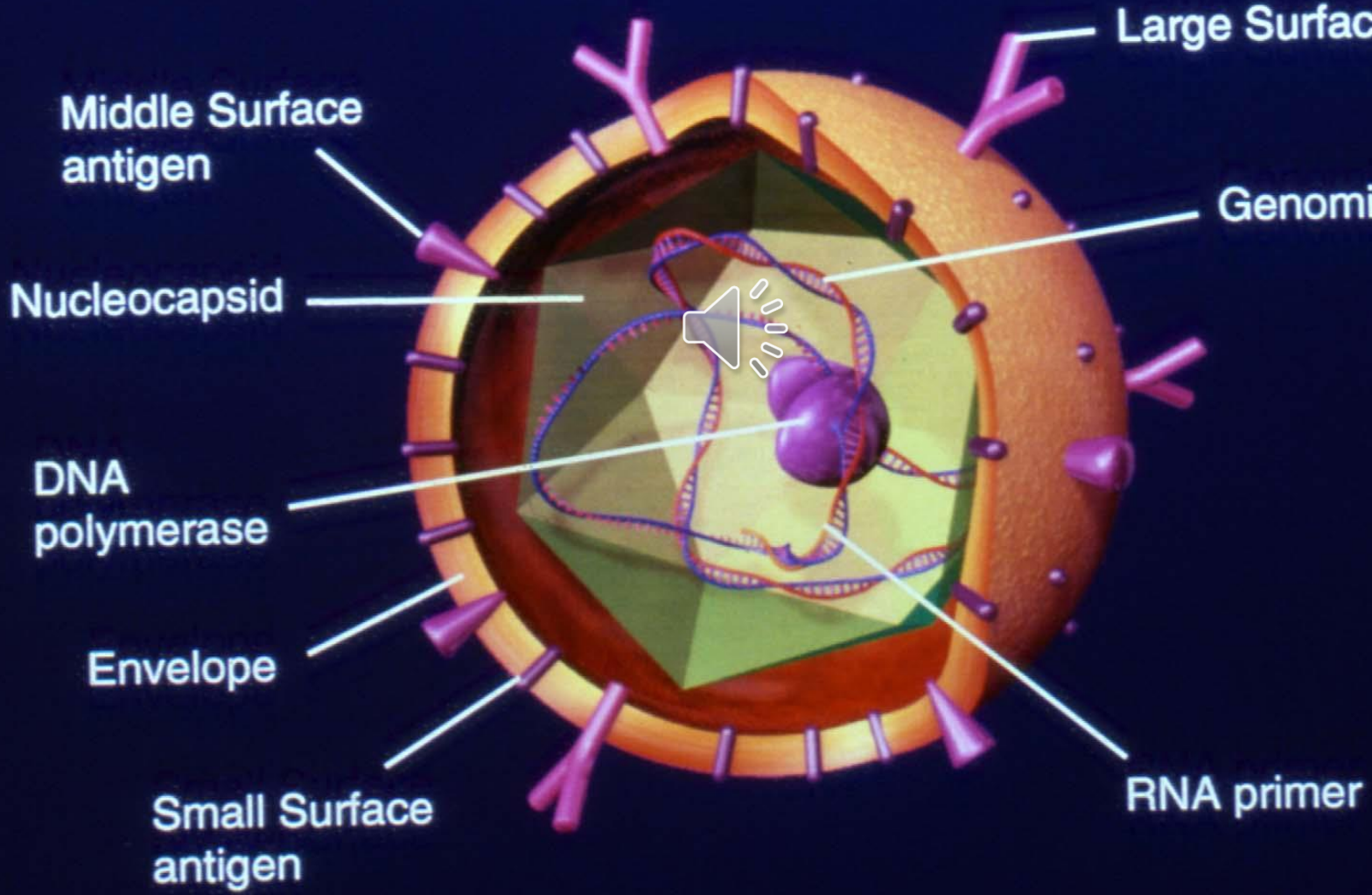
Lift arm slightly out to side.
Bursa will slide underneath the acromion for protection.



Transmission of HBV Infection



Hepatitis B Virus



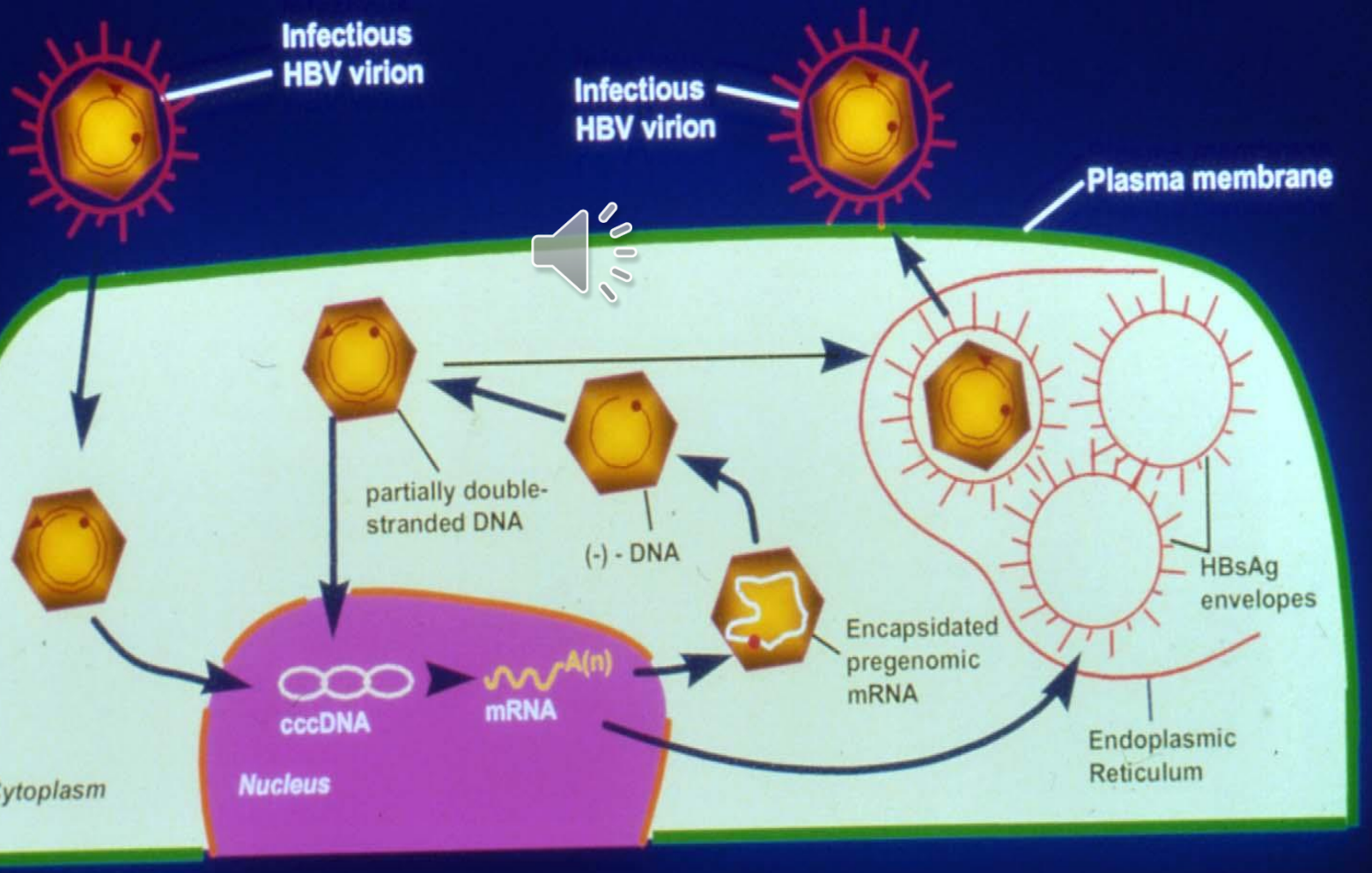
Prevalence of Chronic HBV Carriers

Percentage Chronic HBsAg Carriers:

<2% - Low 2-7% - Intermediate >8% - High

Source: U.S. Centers for Disease Control & Prevention

Replication Cycle of the Hepatitis B Virus



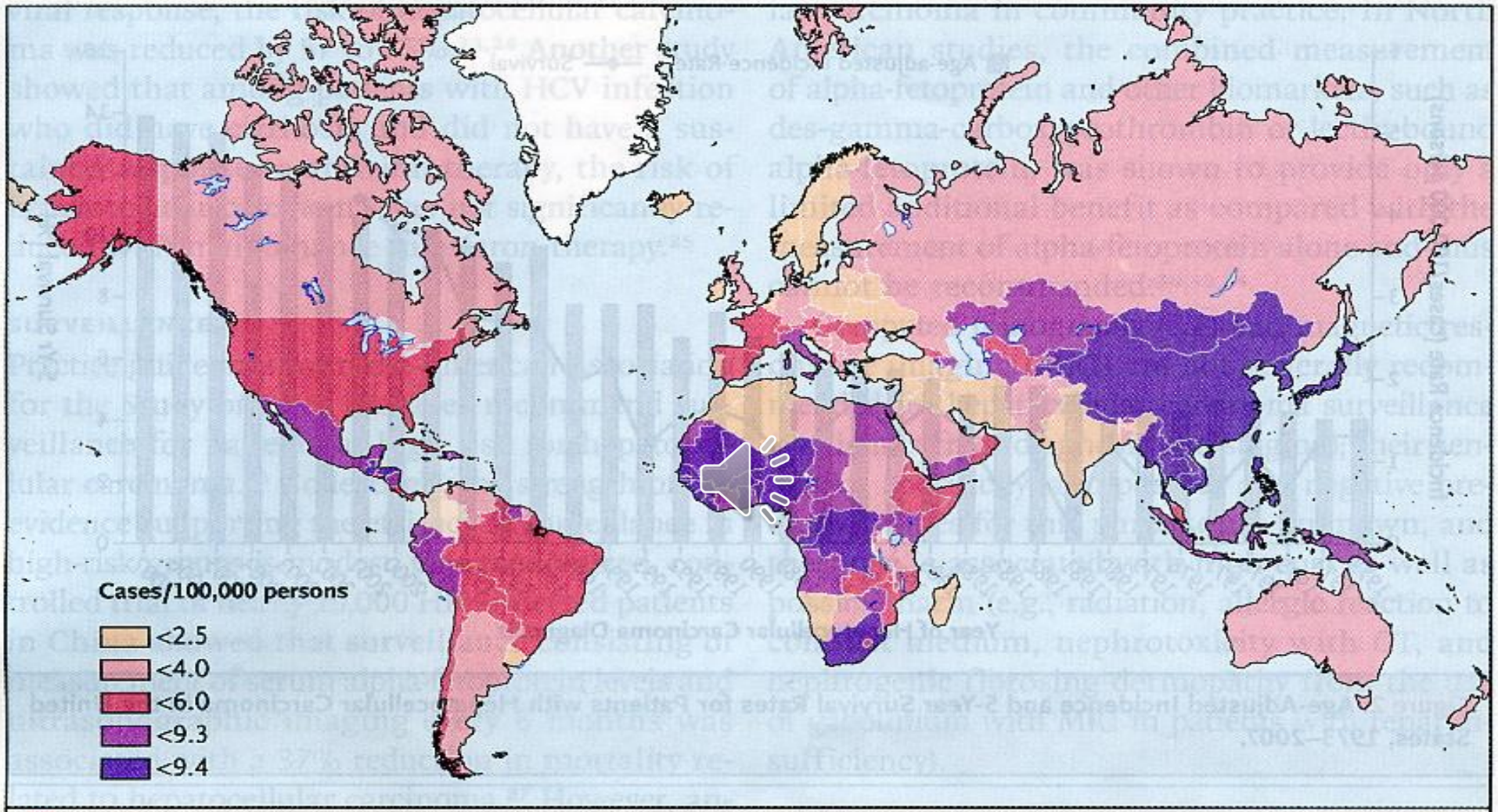
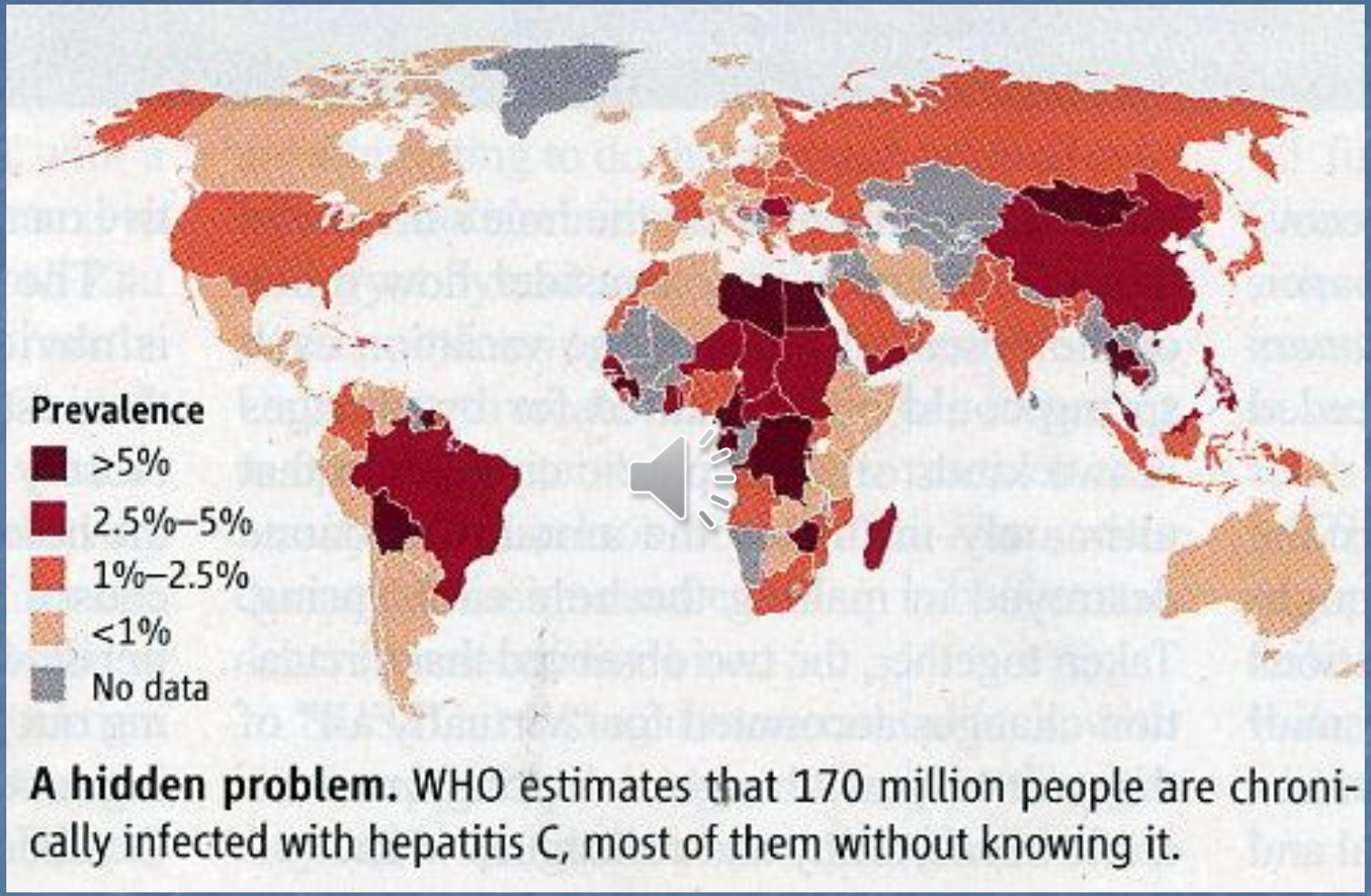
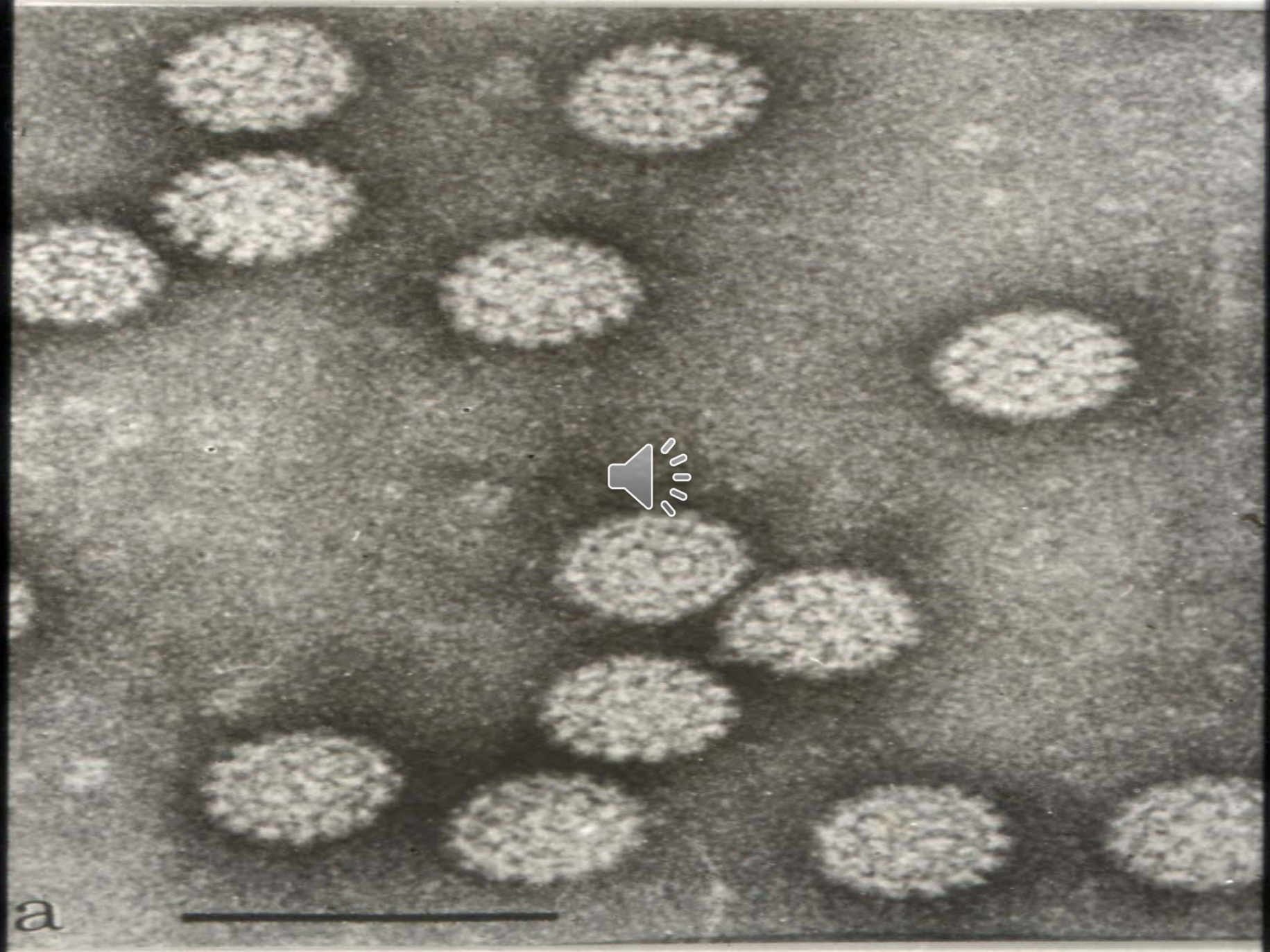


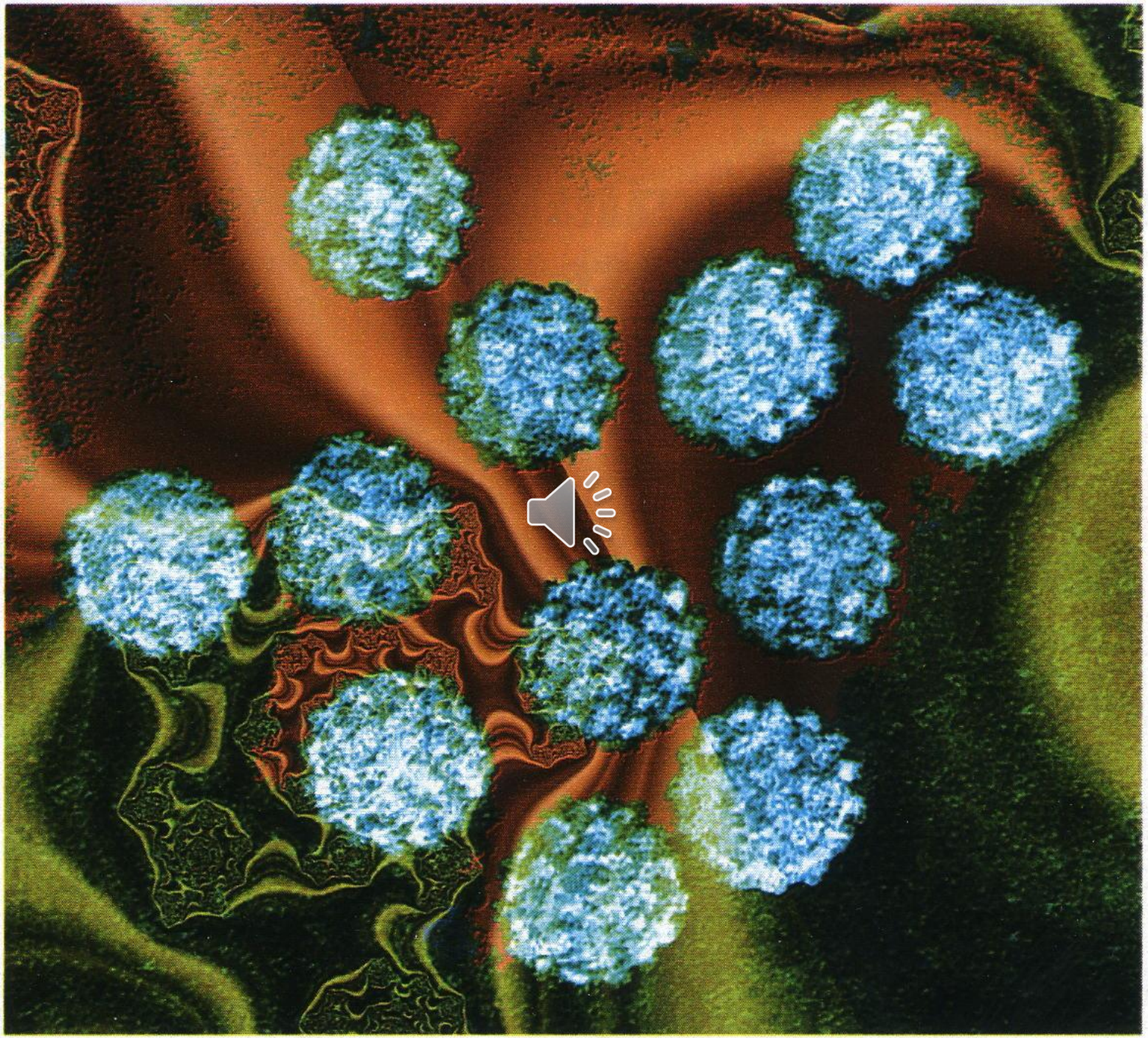
Figure 1. Regional Variation in the Estimated Age-Standardized Incidence Rates of Liver Cancer.

The incidence rates shown (numbers of cases per 100,000 persons) pertain to both sexes and all ages. Adapted from the World Health Organization.[3]

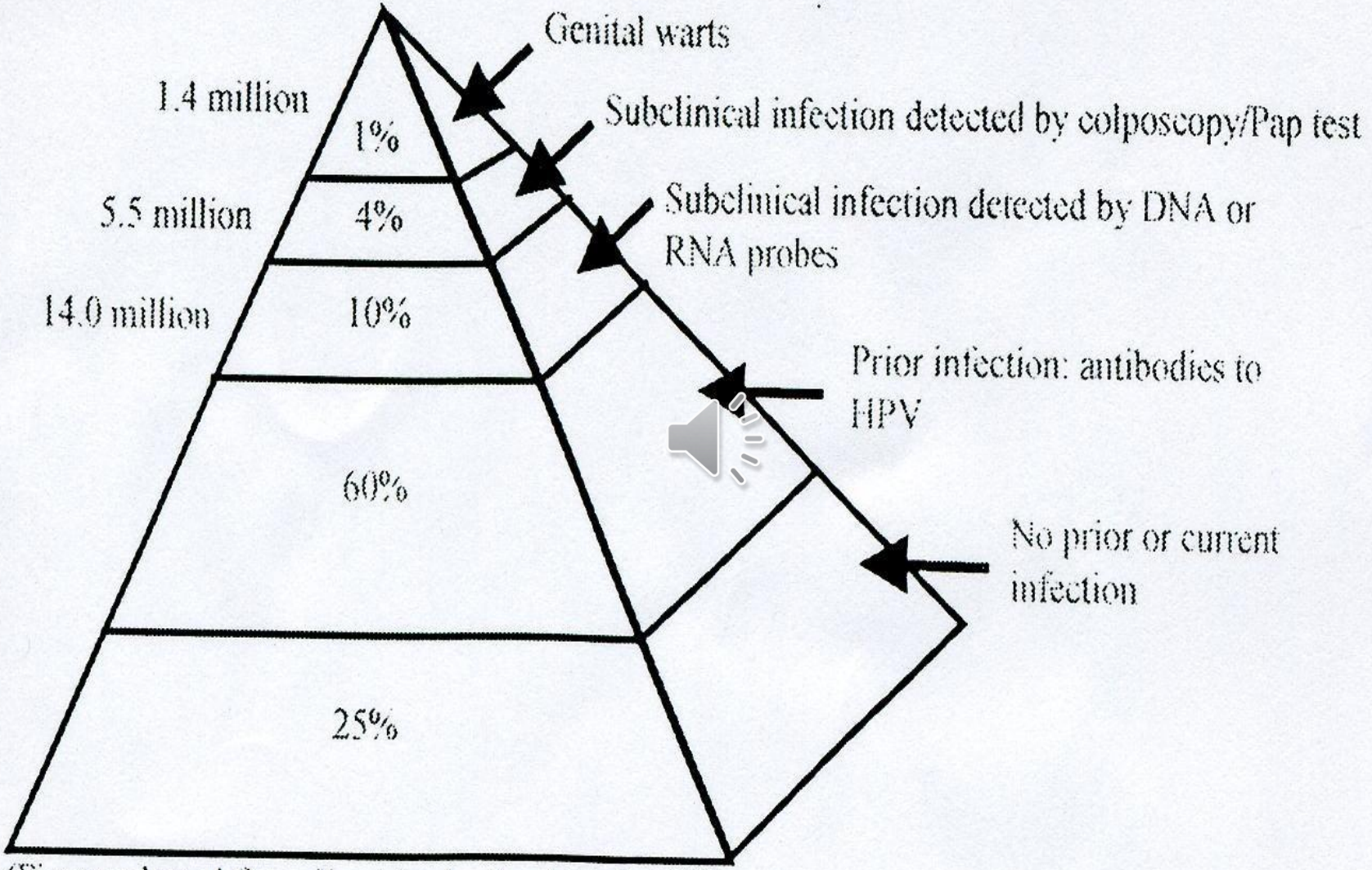


A hidden problem. WHO estimates that 170 million people are chronically infected with hepatitis C, most of them without knowing it.

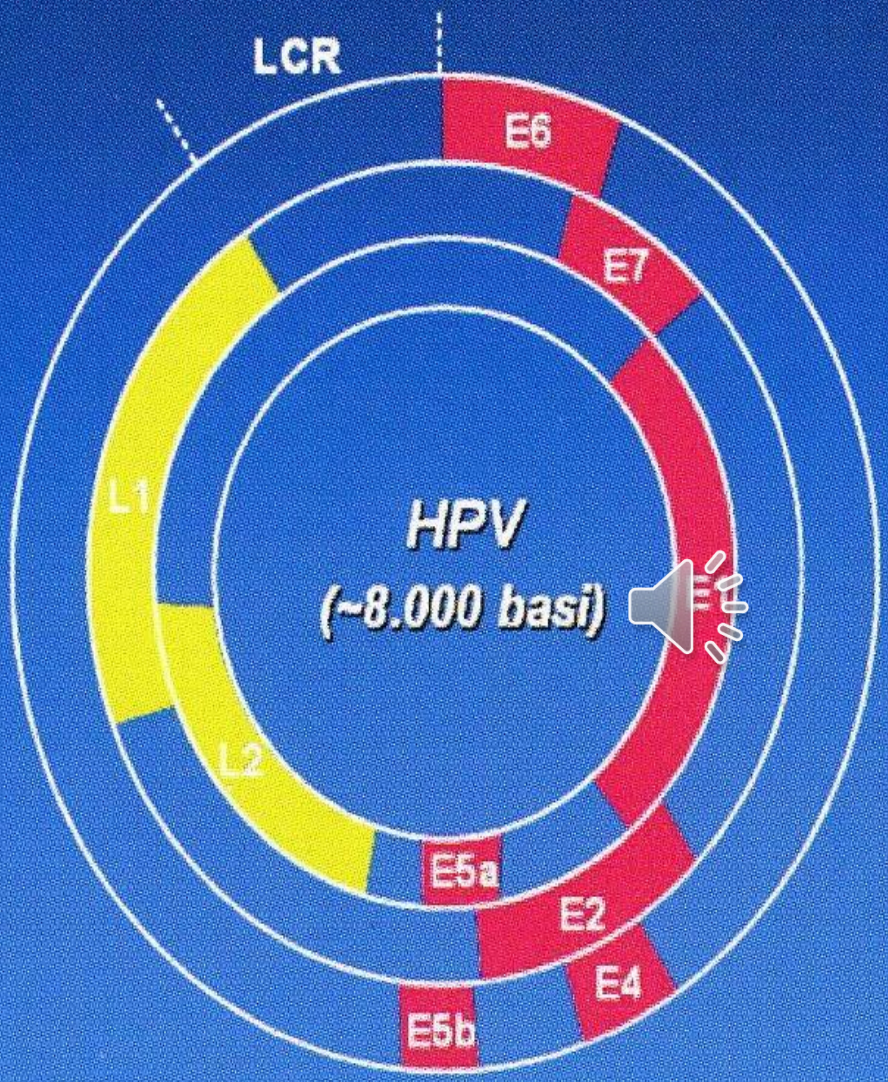

a



Genital HPV infection in the U.S. sexually active population (age 15 to 49)



(Figure adapted from Koutsky L. Am J Med, 1997)

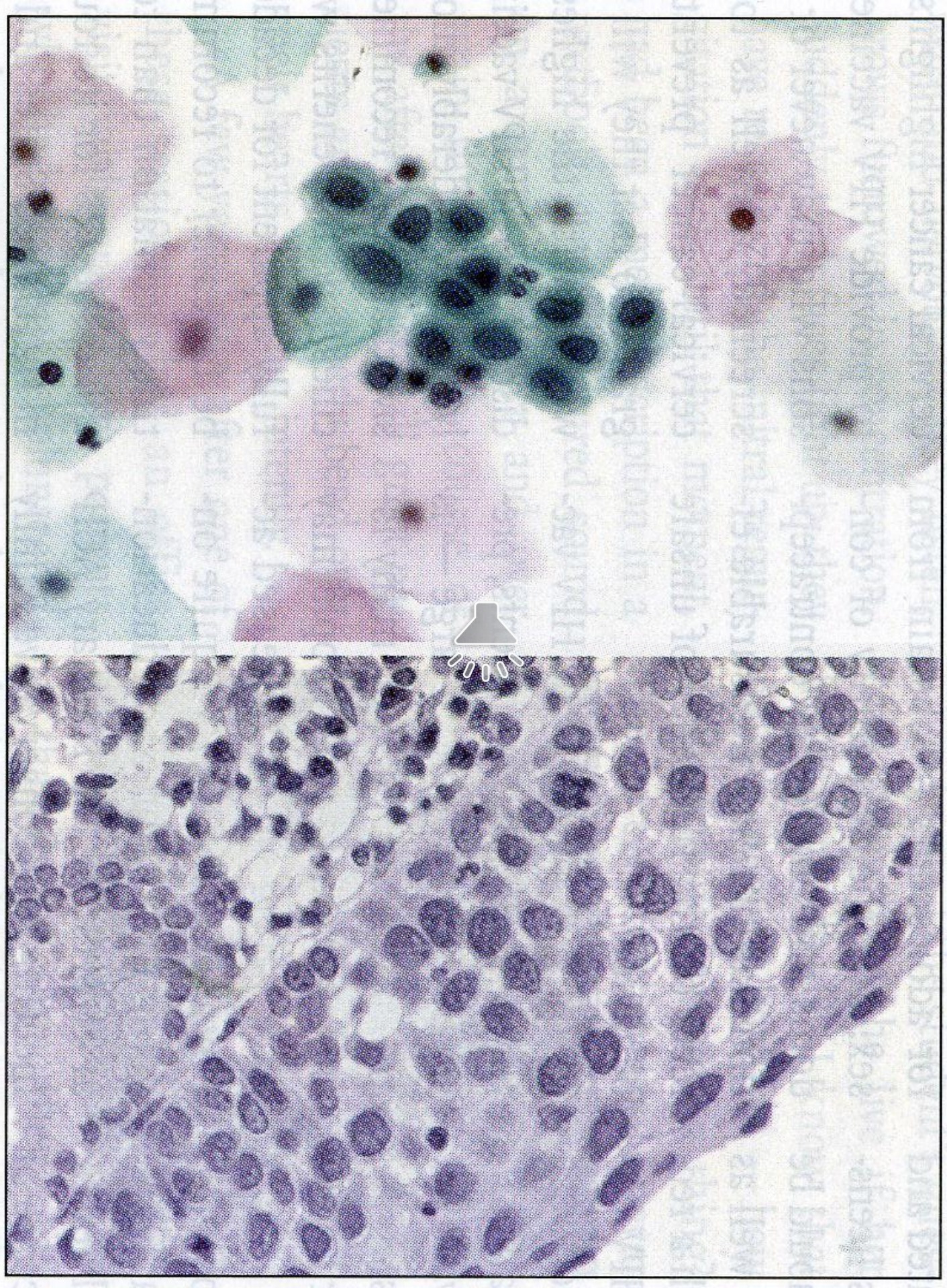


E - early

L - late

LCR - long control region

Phelps WC et al., Ann Intern Med 1995



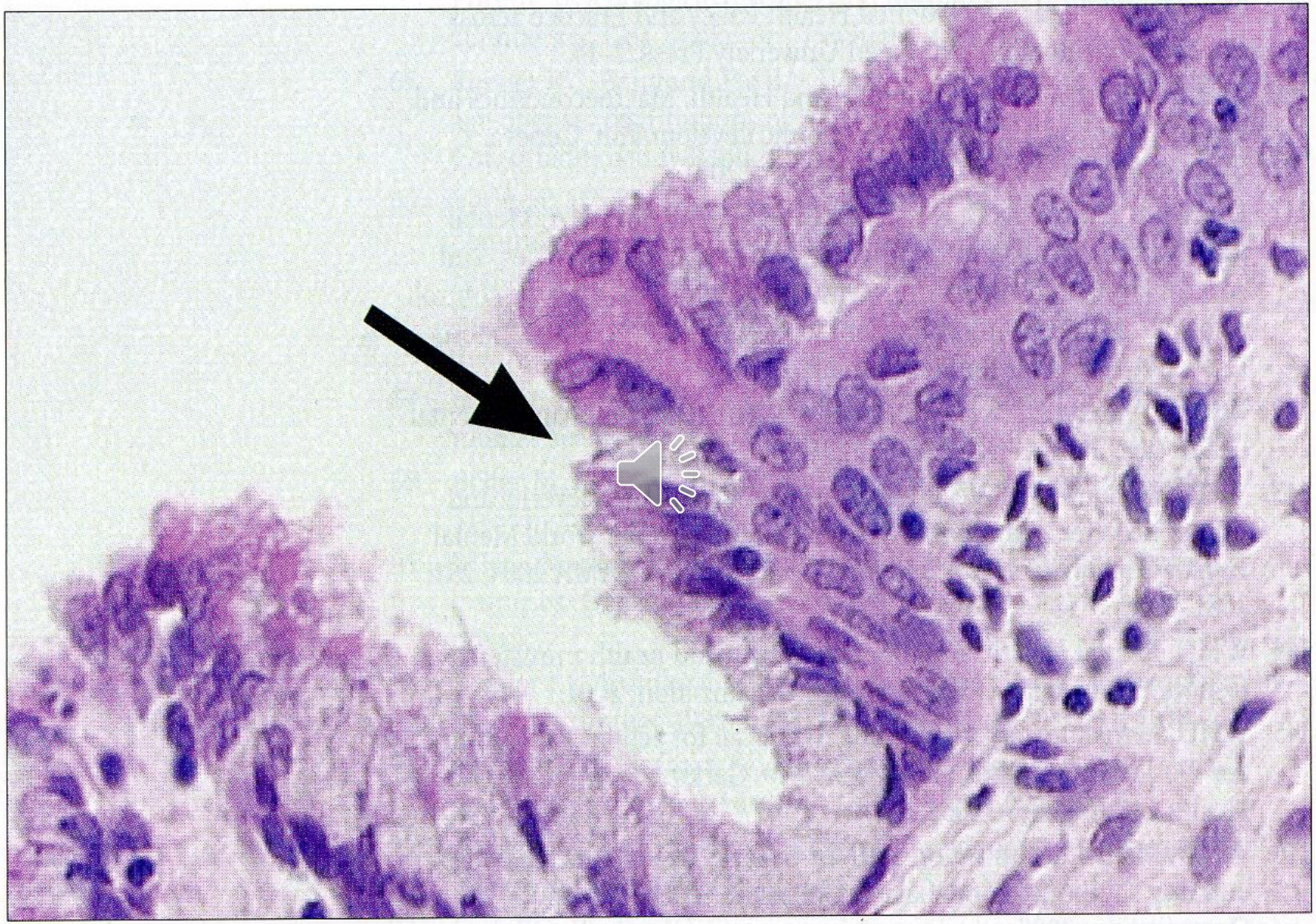


Figure 1: The cervical transformation zone

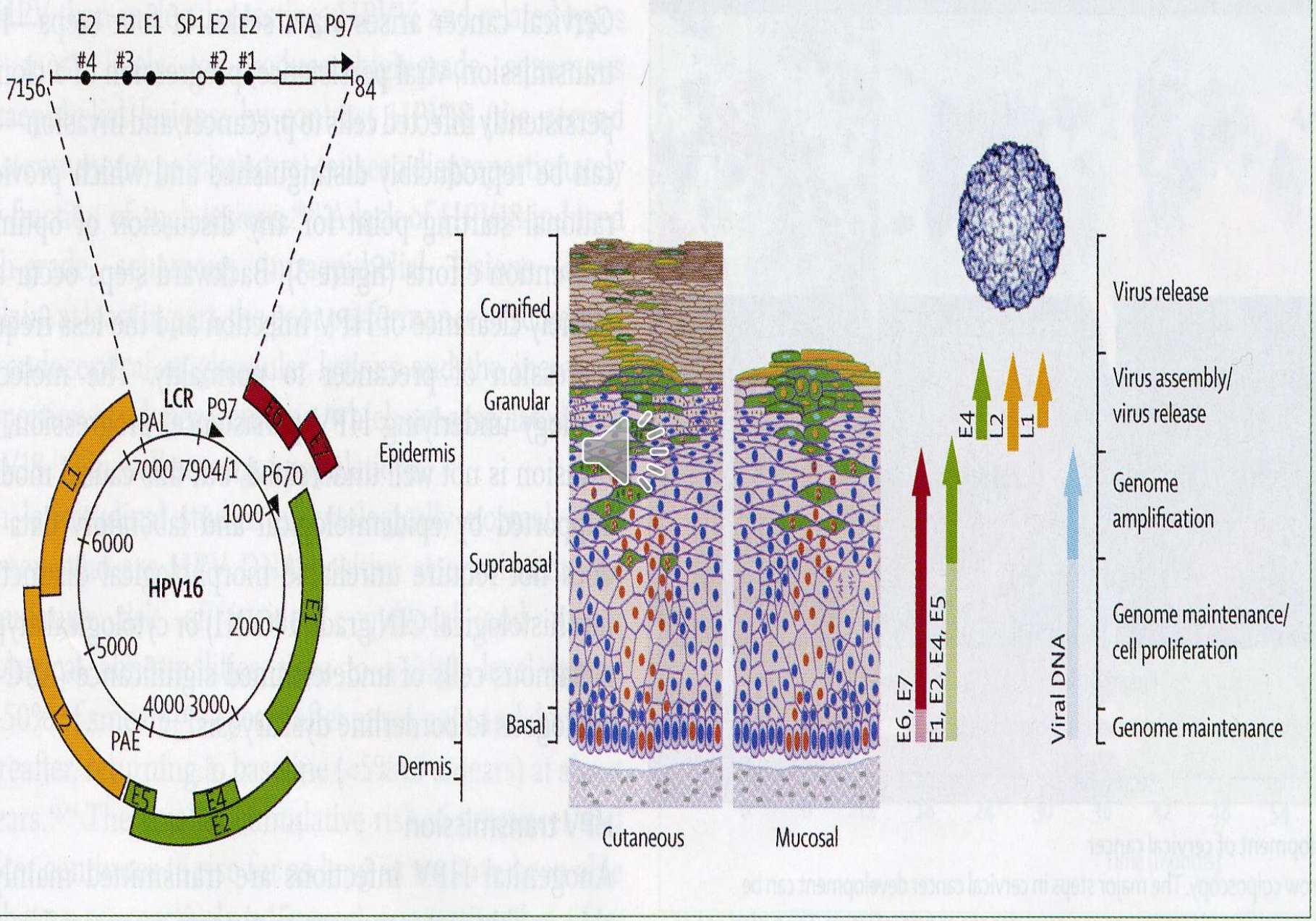


Figure 2: The HPV genome and its expression within the epithelium

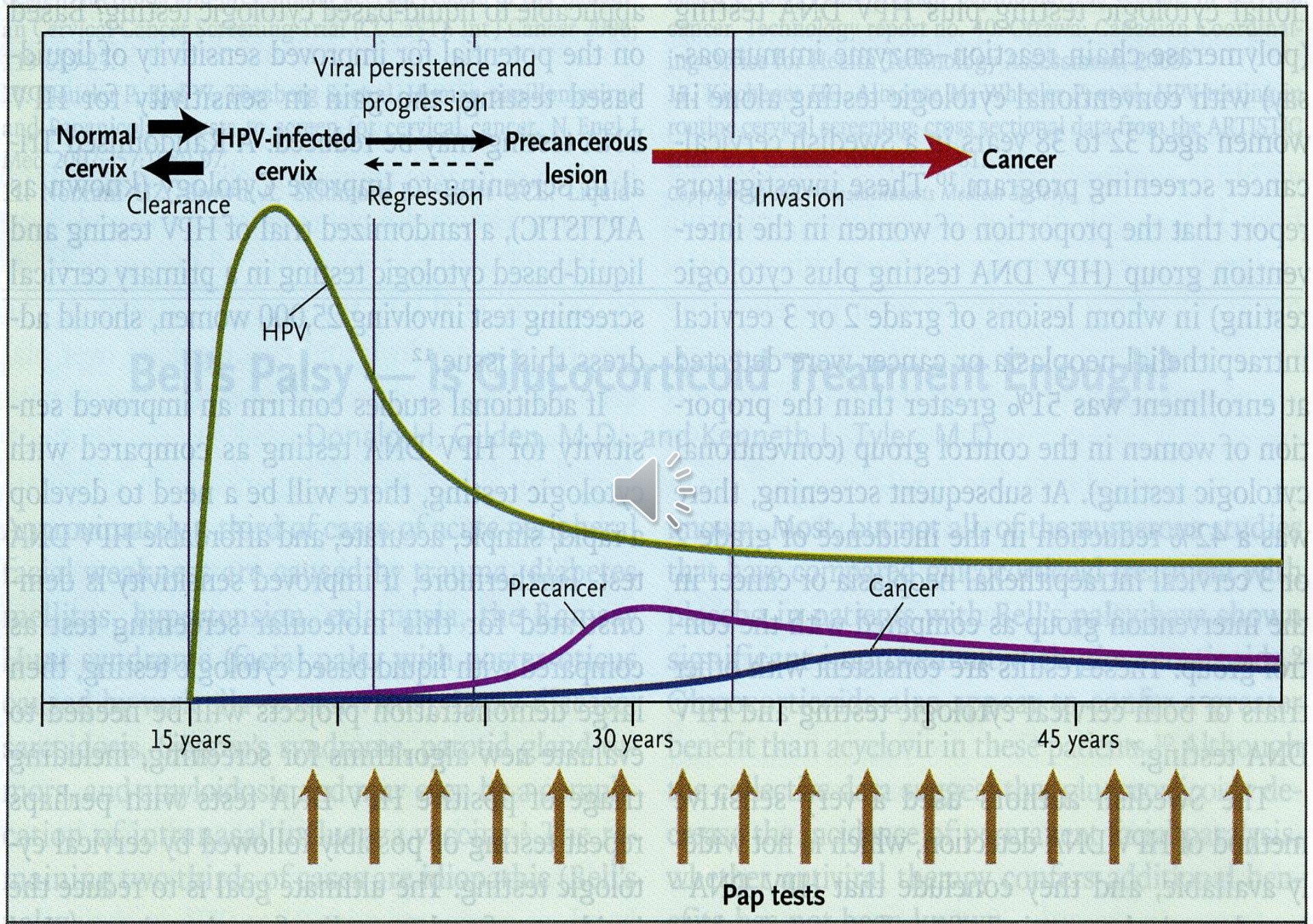


Figure 1. Natural History of HPV Infection and Cervical Cancer.

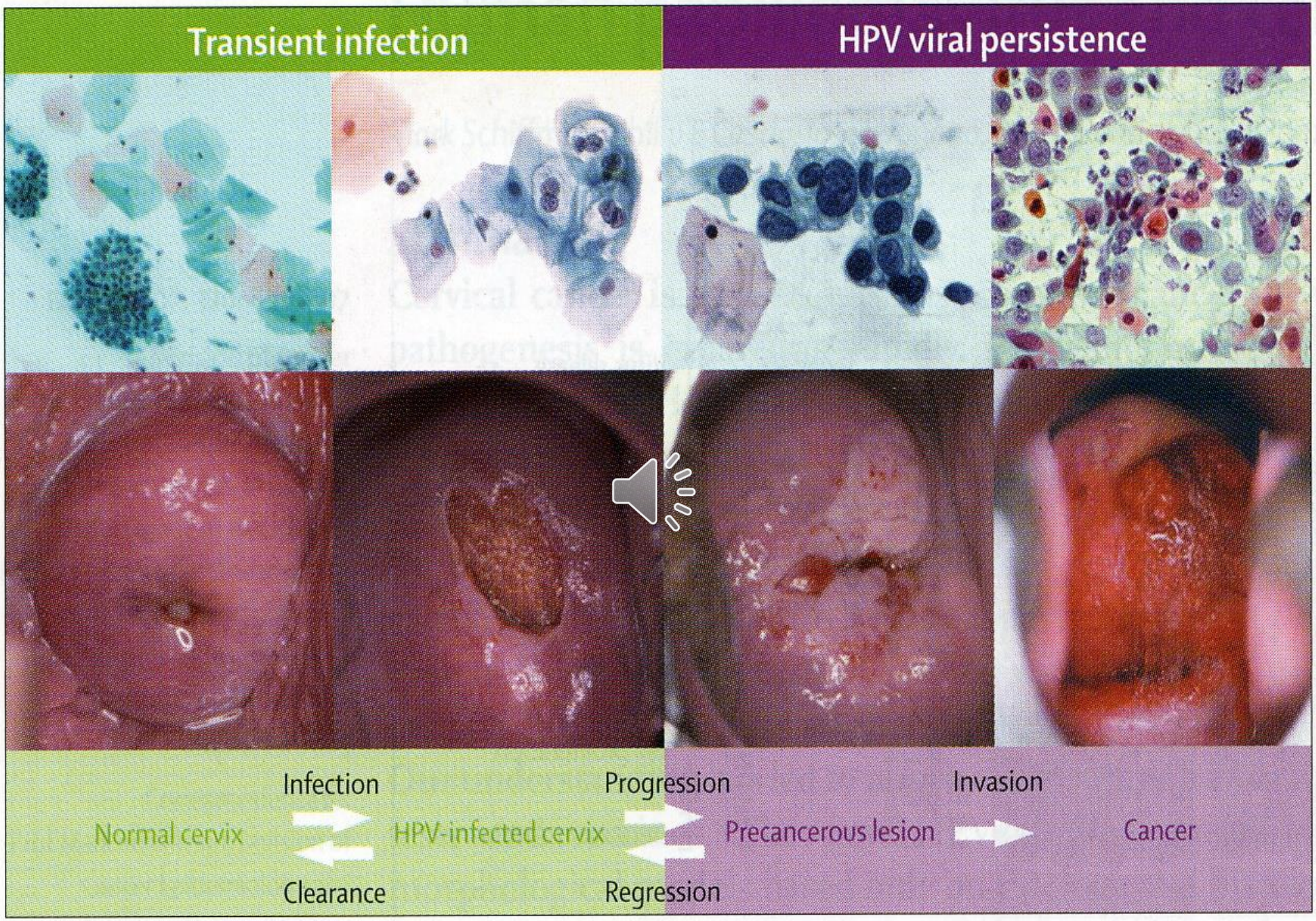
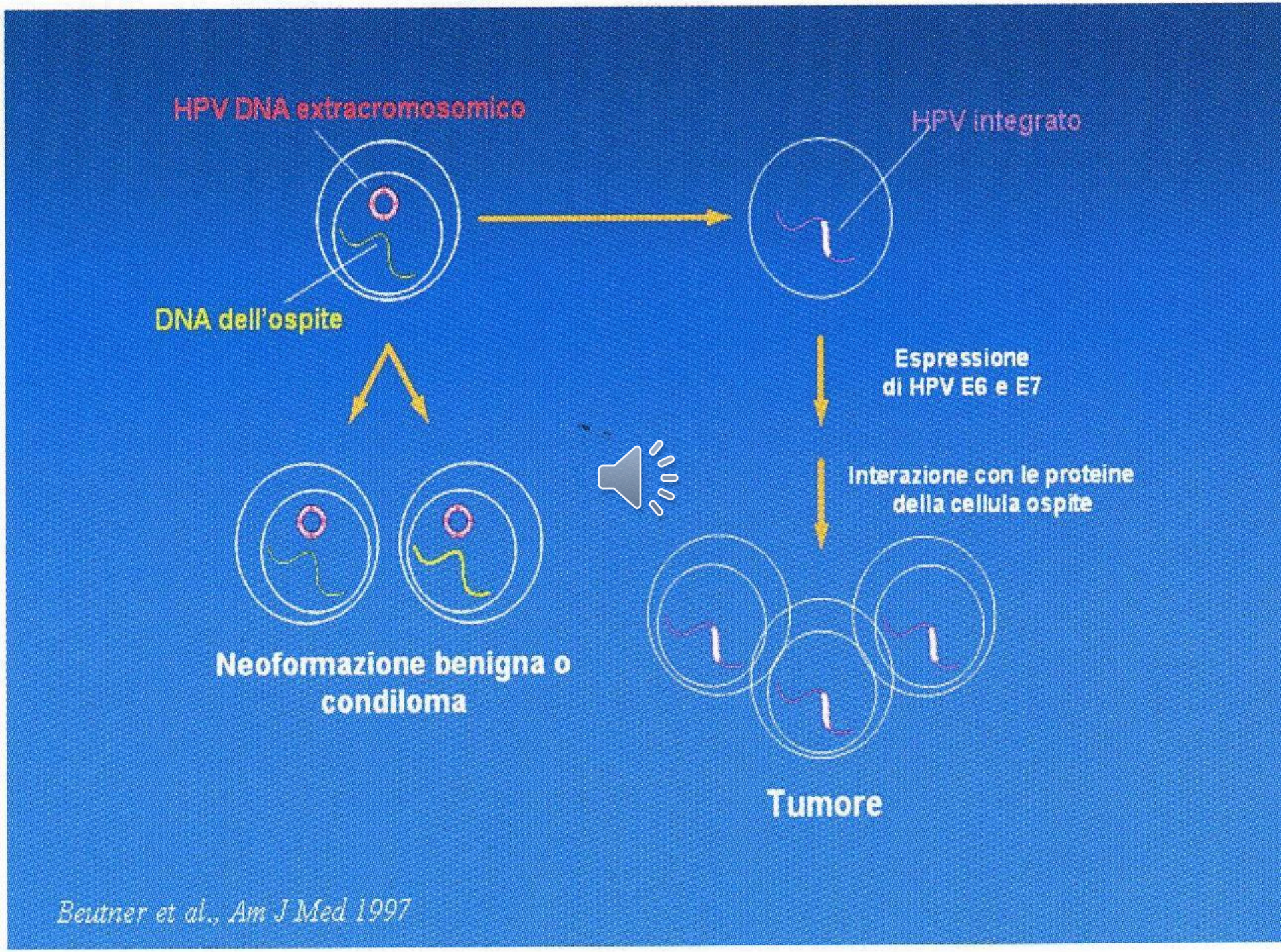


Figure 3: **Major steps in the development of cervical cancer**



Beutner et al., Am J Med 1997

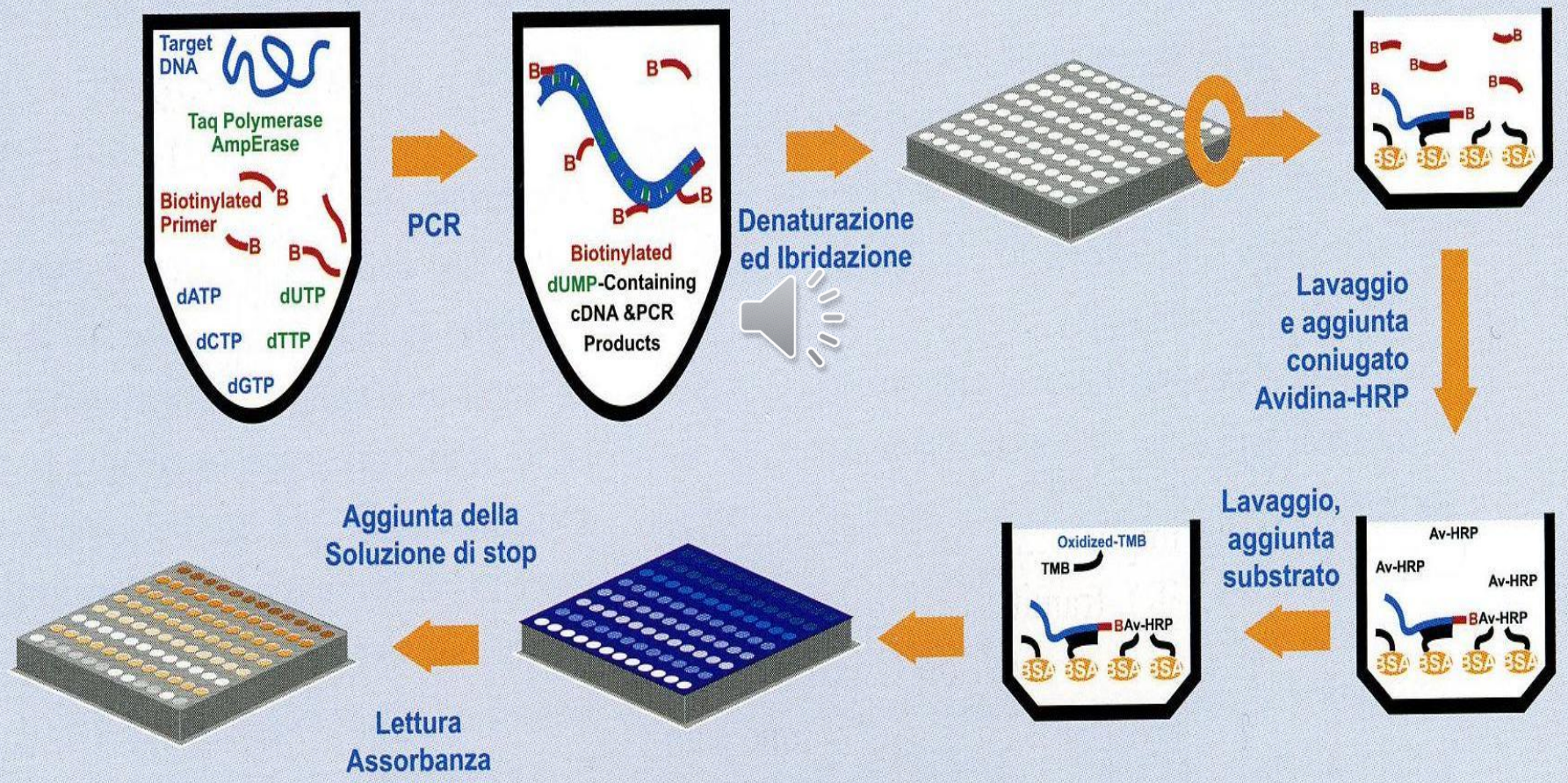

COSÌ LA PROTEINA IMPAZZITA S'ALLEA CON IL TUMORE
INTERAZIONE P53 MUTATA-P73
CELLULA TUMORALE
FARMACI ANTITUMORALI
P73
IL SEQUESTRO
La proteina P53 mutata sequestra e inattiva proteine (P73) coinvolte nella risposta al farmaco anti-tumorale, impedendo così la morte della cellula maligna.
P53 MUTATA
PROTEINE NFY
FARMACI ANTITUMORALI
LA MOLTIPLICAZIONE
In risposta ai farmaci anti-tumorali la P53 mutata favorisce la produzione e l'attivazione di proteine (NFY), che hanno attività pro-tumorigenica.
P53 MUTATA
ONCO-MIR
ANTI-TUMORALI
ONCOSOPPRESSORE INATTIVATO
I MICRO-RNA
In risposta a farmaci anti-tumorali la proteina P53 mutata promuove l'espressione degli onco-Mir, piccoli Rna in grado di impedire l'attivazione di geni antitumorali.

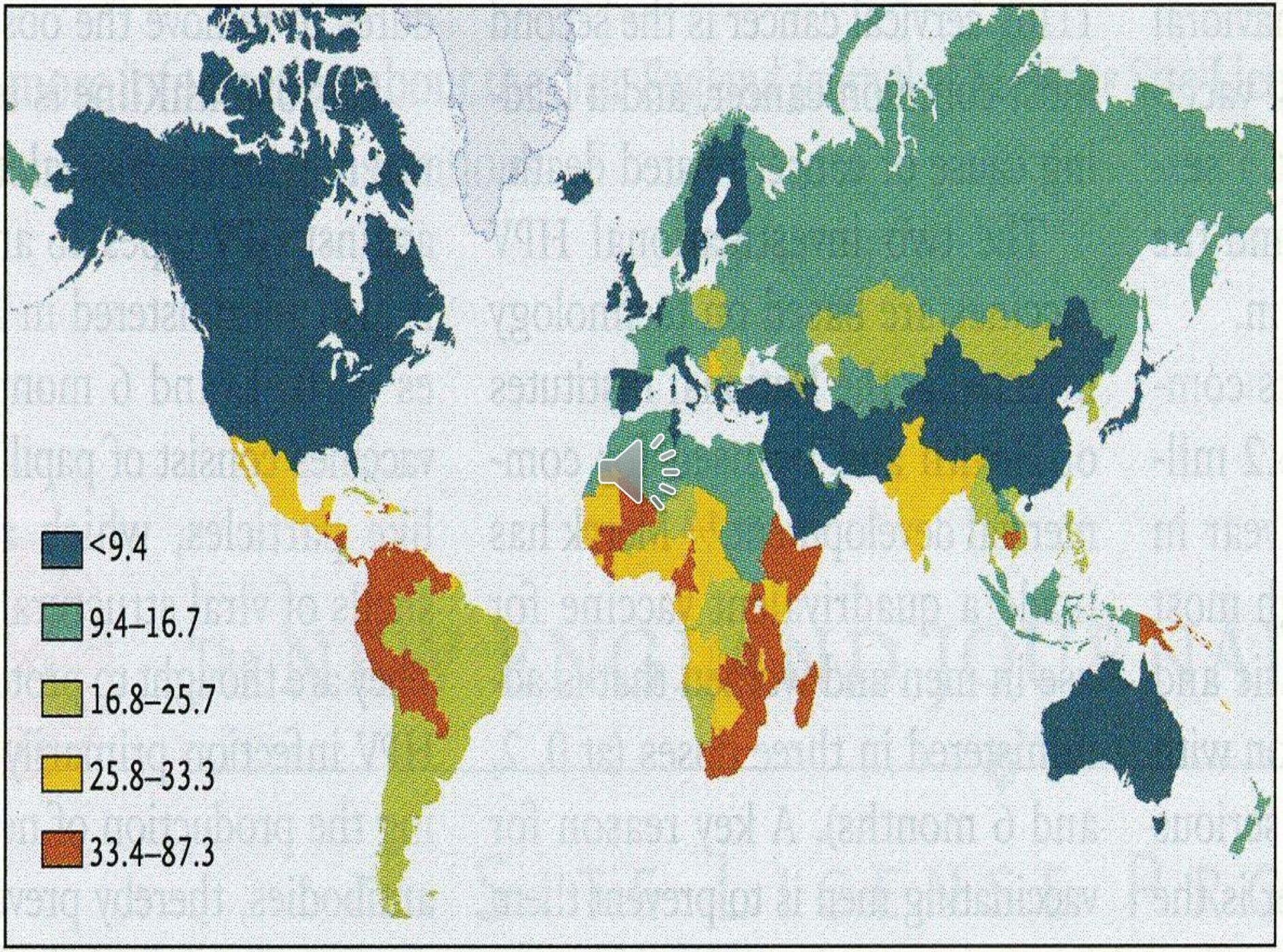
	Proportion of cervical cancers caused	Cumulative total
HPV16	54·6%	54·6%
HPV18	15·8%	70·4%
HPV33	4·4%	74·8%
HPV45	3·7%	78·5%
HPV31	3·5%	82·0%
HPV58	3·4%	85·4%
HPV52	2·5%	87·9%
HPV35	1·8%	89·7%
HPV59	1·1%	90·8%
HPV56	0·8%	92·2%
HPV51	0·7%	92·9%
HPV39	0·7%	93·6%
HPV73	0·5%	94·1%
HPV68	0·5%	94·6%
HPV82	0·2%	94·8%
No type identified	5·2%	**100%**

Data adapted from reference 18.

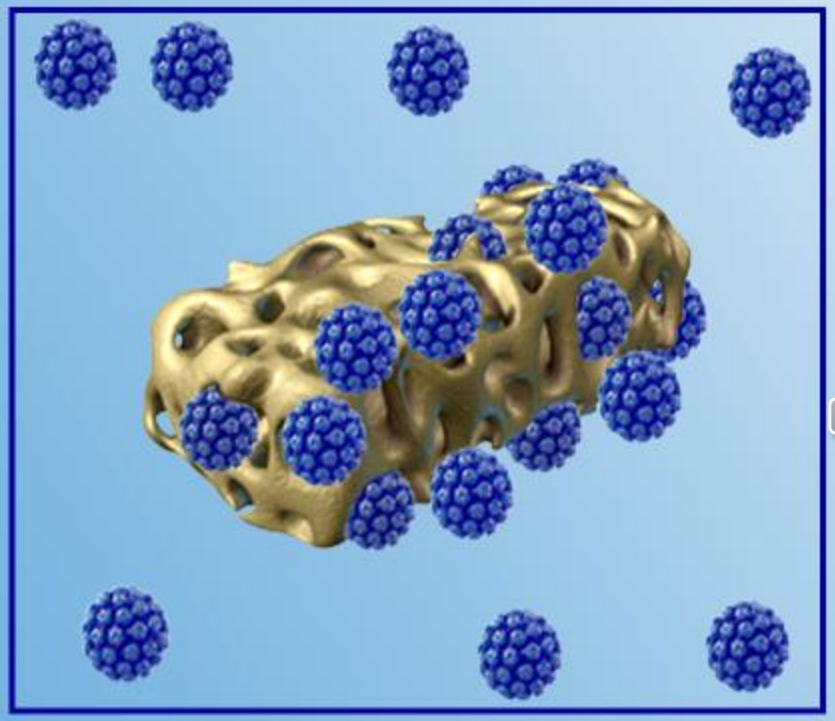
Table 2: **Proportion of cervical cancer caused by the carcinogenic HPV types**

Schema della metodica Amplicor HPV test

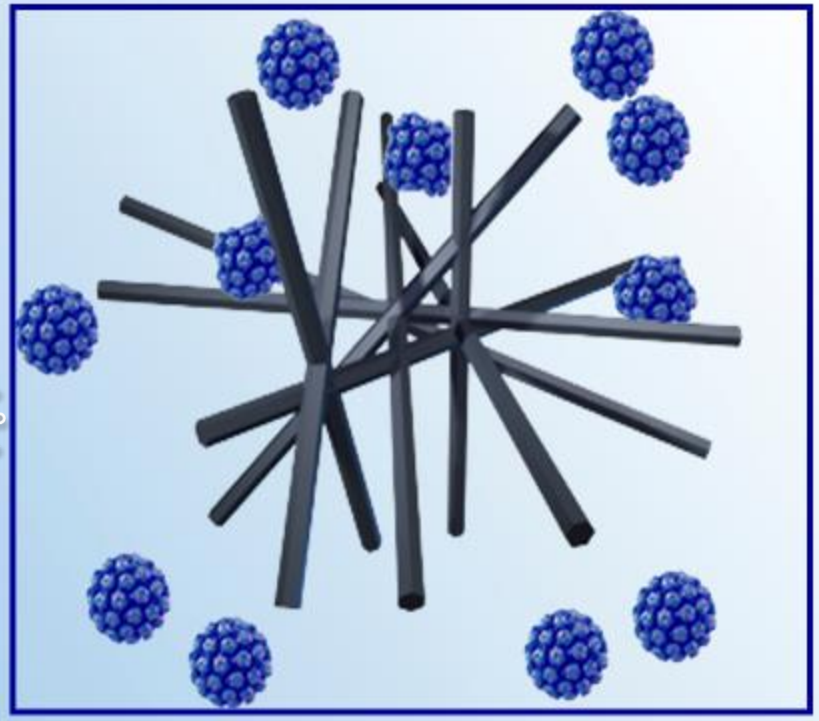



<9.4
9.4–16.7
16.8–25.7
25.8–33.3
33.4–87.3

AAHS* e legame con le VLP



AAHS*



$Al(OH)_3$**

La capacità di legare le particelle del vaccino in modo stabile è importante.

AAHS*è in grado di stabilizzare il legame delle particelle virali in modo più stabile rispetto all'idrossido di alluminio.

*Alluminio Idrossifosfato Solfato Amorfo

** Idrossido di Alluminio

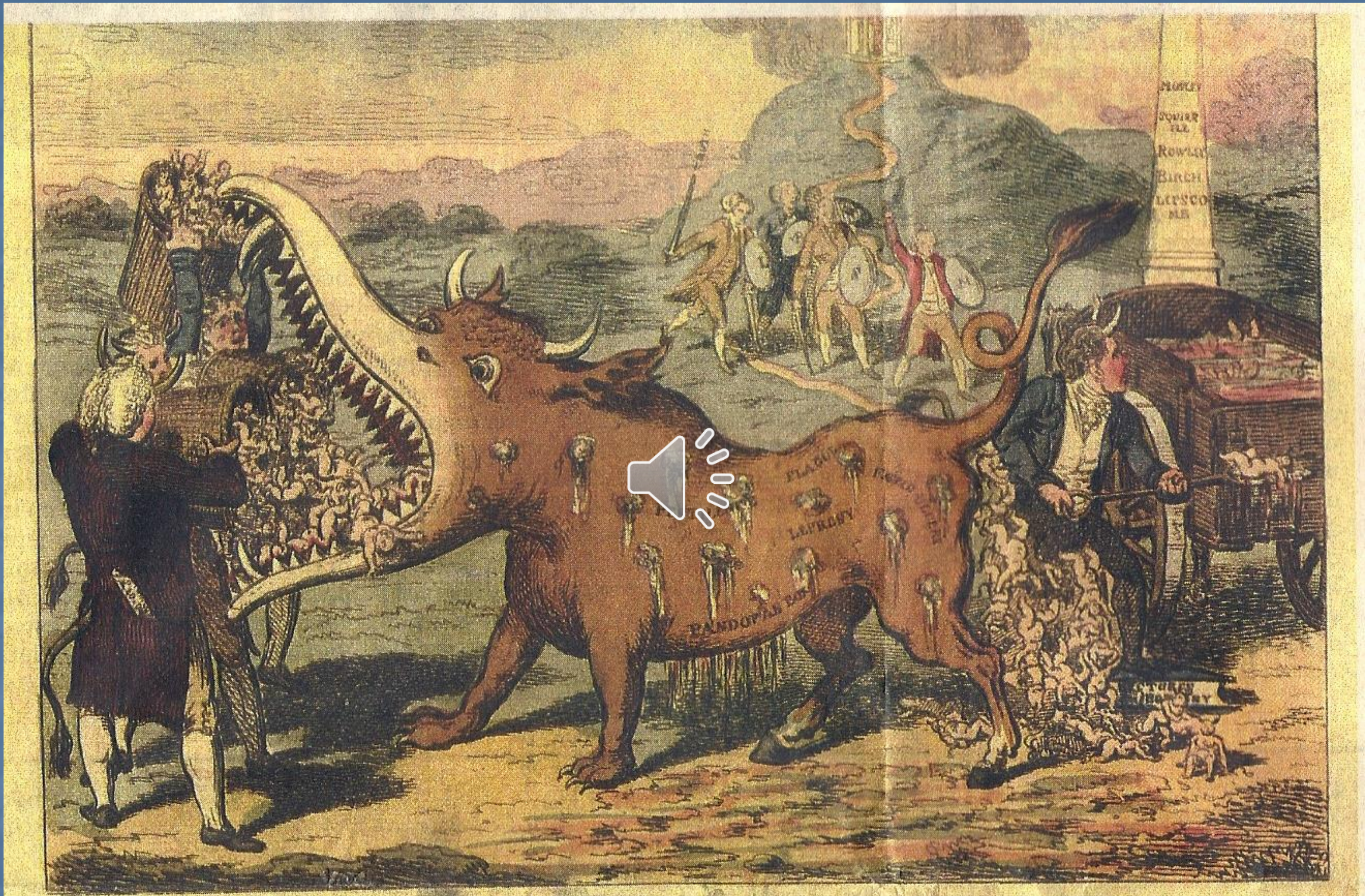
| *Caulfield MJ, Shi L, Wang S et al. Vaccine 2007*

VACCINI PER L'HPV

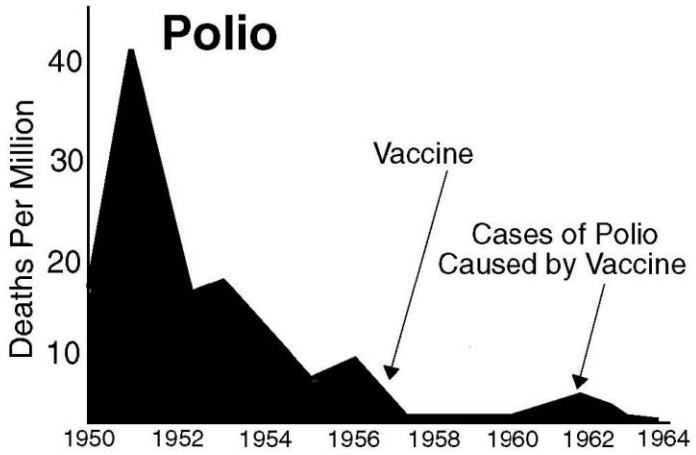
FARMACO*	TIPI HPV	FORMULAZIONI	DOSE/SCHEDULA	COSTO (EURO)[1]
Cervarix-GlaxoSmithKline	16 e 18	siringhe monodose da 0,5 ml[2]	0,5 ml IM/3 dosi (0, 1 e 6 mesi)	470,37
Gardasil-Sanofi	6, 11, 16 e 18	siringhe monodose da 0,5 ml[3]	0,5 ml IM/3 dosi (0, 2 e 6 mesi)	514,92
Gardasil 9[4] (Sanofi)	6, 11, 16, 18, 31, 33, 45, 52 e 58		0,5 ml IM/3 dosi (o, 2 e 6 mesi)	n.d.

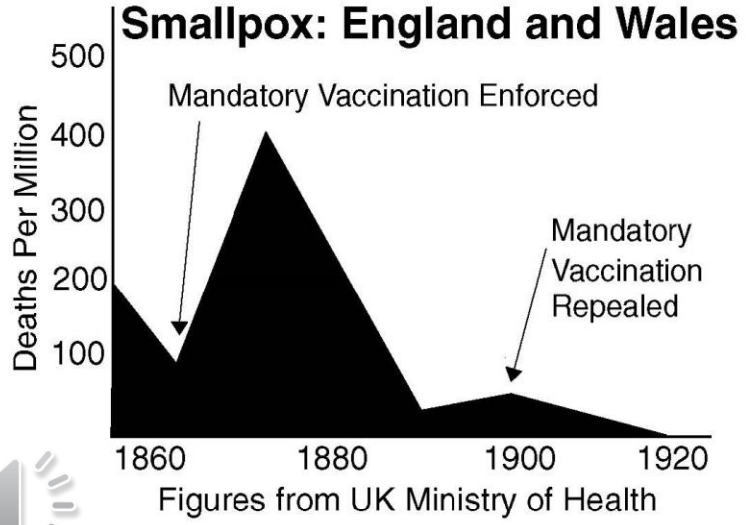
*Possono essere disponibili altri medicinali contenenti gli stessi principi attivi.

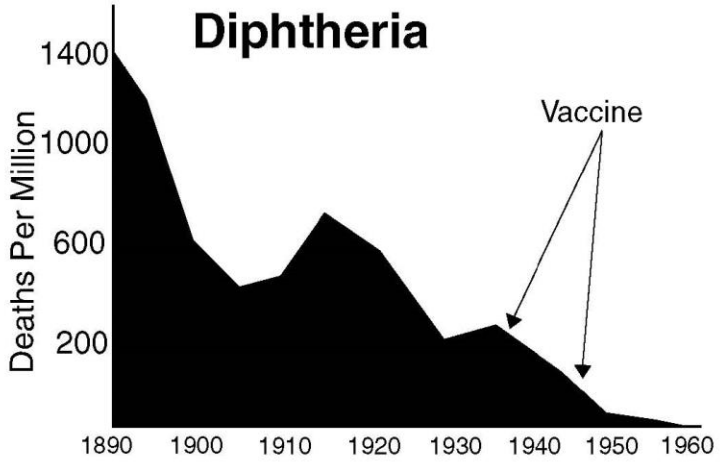
1. Farmaco ospedaliero esitabile. Costo all'ospedale per un ciclo vaccinale (3 dosi).
2. Venduto in confezioni da 1 o 10 siringhe monodose preriempite.
3. Venduto in confezioni da 1 siringa monodose preriempita.
4. Il farmaco ha ricevuto il parere positivo del CHMP in data 26 marzo 2015; non disponibile in commercio in Italia.

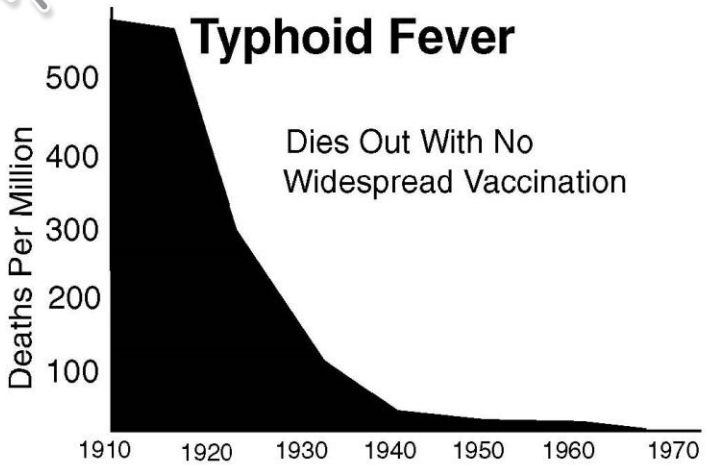


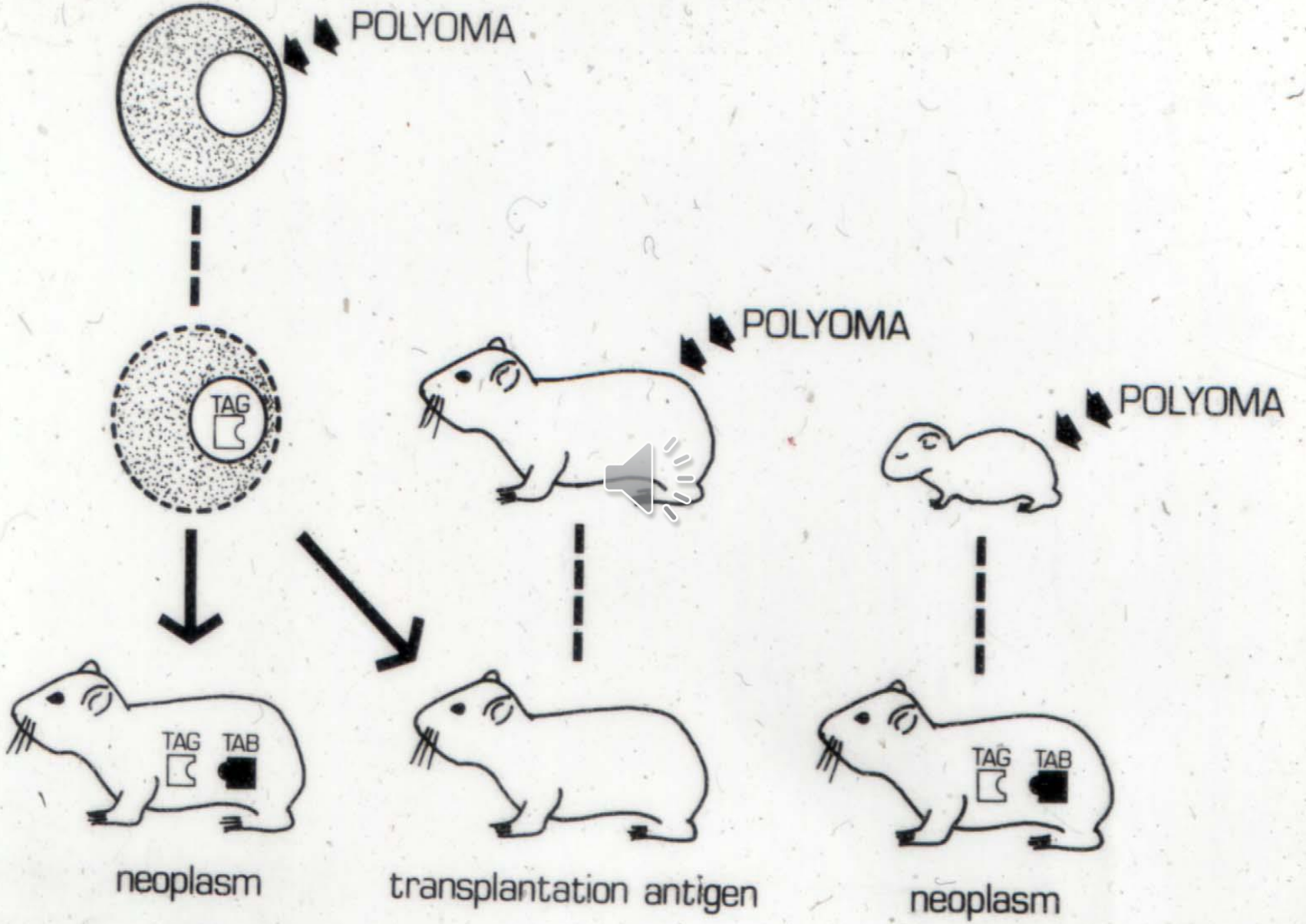
VACCINATION.


Polio
Deaths Per Million
40
30
20
10
Vaccine
Cases of Polio
Caused by Vaccine
1950
1952
1954
1956
1958
1960
1962
1964

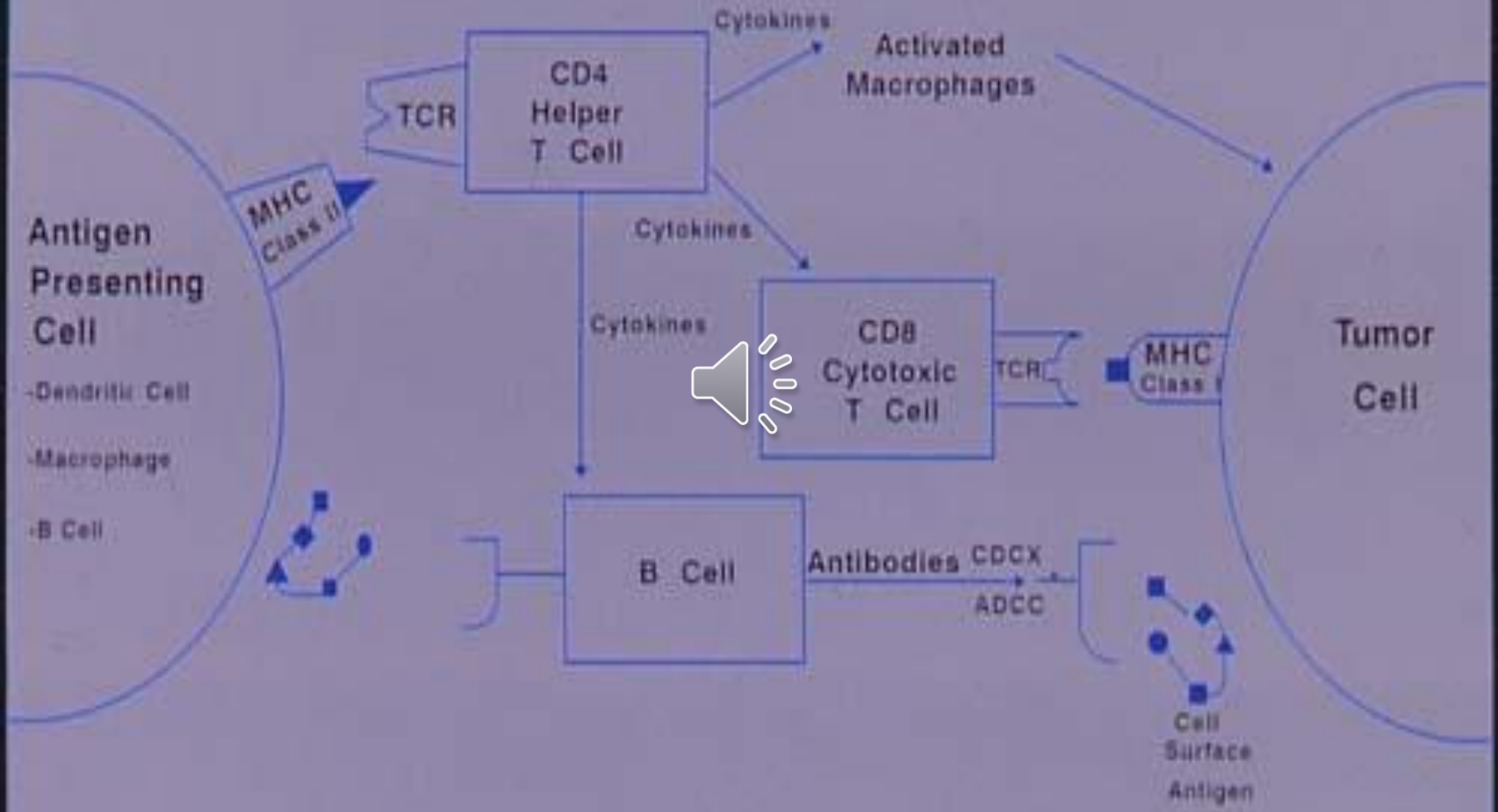

Smallpox: England and Wales
Deaths Per Million
500
400
300
200
100
Mandatory Vaccination Enforced
Mandatory
Vaccination
Repealed
1860
1880
1900
1920
Figures from UK Ministry of Health


Diphtheria
Deaths Per Million
1400
1000
600
200
Vaccine
1890
1900
1910
1920
1930
1940
1950
1960


Typhoid Fever
Deaths Per Million
500
400
300
200
100
Dies Out With No
Widespread Vaccination
1910
1920
1930
1940
1950
1960
1970


POLYOMA
TAG
POLYOMA
POLYOMA
TAG
TAB
TAG
TAB
TAG
TAB
neoplasm
transplantation antigen
neoplasm

Mechanisms for tumor recognition and rejection



Source: Annals N.Y. Academy of Sciences

Long years of research were required for boosting the immune system to fight cancer.

1890s - Mixtures of dead bacteria were injected by William B. Coley into cancer patients to stimulate the immune system.

1909 - According to Paul Ehrlich the immune system may suppress tumor development.

1960s – Both in animals and men neoplastic cell antigens stimulate the onset of specific humoral and cellular antibodies

1972 - Immunogenecity of a soluble transplantation antigen from adenovirus 12 - induced tumor cells demostrated in inbred hamsters (PD-4). Ariel Hollinshead et al., Can. J. Microbiol 18;1365-1369.

1975 – Discovery of Monoclonal Antibodies, highly specific immunological tools

1980 – Mass-production of interferon, the immune-stimulating molecule, after inserting its coding gene into bacteria

Therapeutic Vaccine Strategies (A)

Tumor cells are removed from a patient and treated biochemically or irradiated.

Then the extracts of the dead cancer cells are reinjceted, boosting the immune system to attack the tumor cell

1983 – Tumor liberated protein (TLP) boosts the immune system's cancer responsive capabilities. G. Tarro et al., Oncology 40:248-254

1986 – Interferon is approved by the Food and Drug Administration (FDA) for the treatment of hairy cell leukemia

1991 – TLP may have the potential to greatly improve the cure rate and/or serve as a lung cancer vaccine

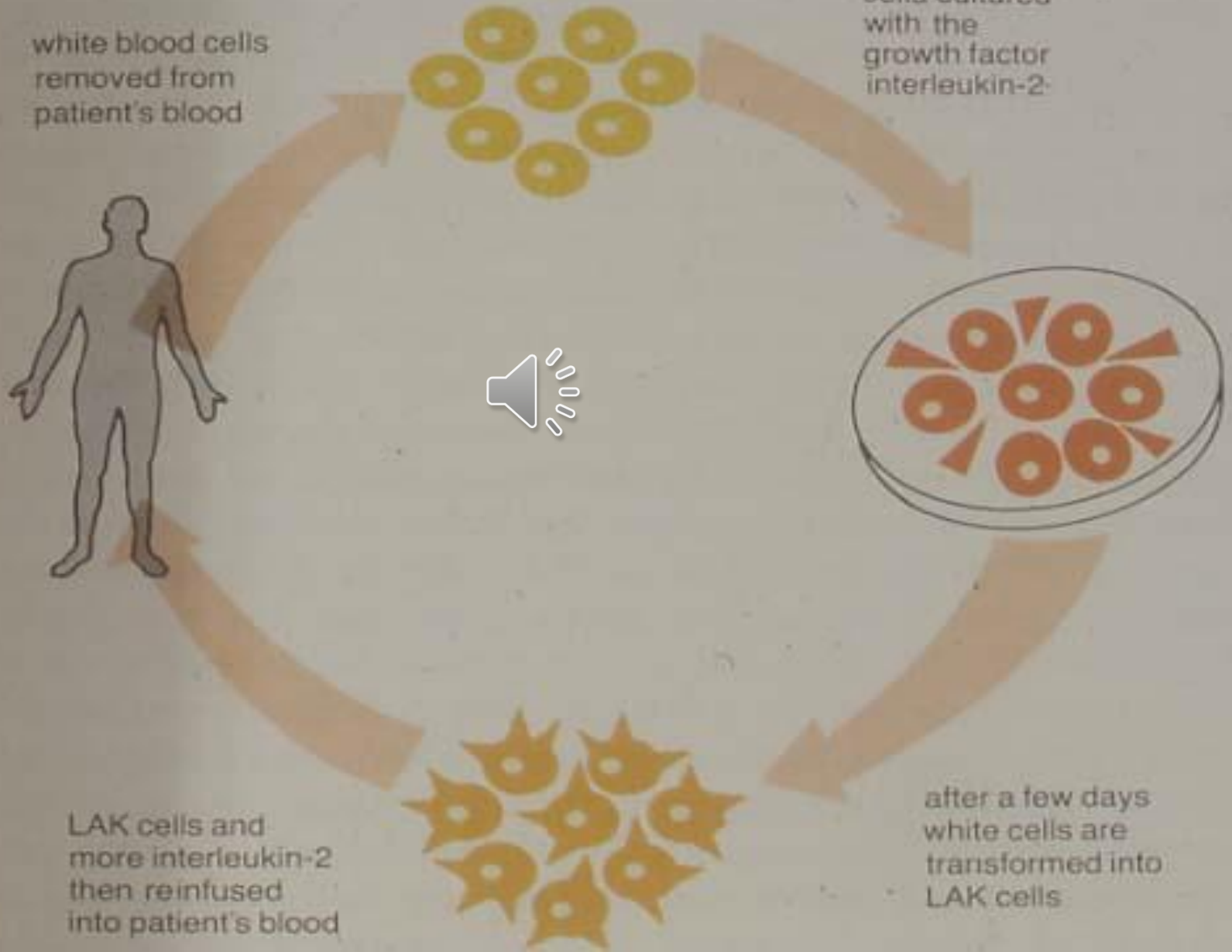
1997 – The FDA okays the first monoclonal antibody (MA) treatment against cancer (for non-Hodgkin's lymphoma)

1998 – The FDA approves the MA Herceptin for the treatment of metastatic breast cancer

2002 – National Cancer Institute researchers prove that two kinds of immune cell – CD4+ T cells and CD8+ T cells-are required for the treatment against cancer.

2002 – Detection of lower levels of TLP/antiTLP may be of clinical relevance (Tarro and Esposito). TLP as candidate marker for the early detection of NSCL cancer

Adoptive Immunotherapy with interleukin-2



Therapeutic Vaccine Strategies (B)

Tumor – associated antigens resulting from protein bits, or from synthesized peptides specific for the cancer tissue, can be used successfully as vaccine to mount a vigorous antitumor attack

2009 – Development of a vaccine approach for therapeutical and preventive application (Giulio Tarro, J. Cell. Physiol. 221: 26-30)

Basic Cellular Immune Response to Cancer

The dendritic cell is an immune cell that presents specific antigens taken from a tumor cell to two other immune cells, the CD4+ and CD8+ cells.

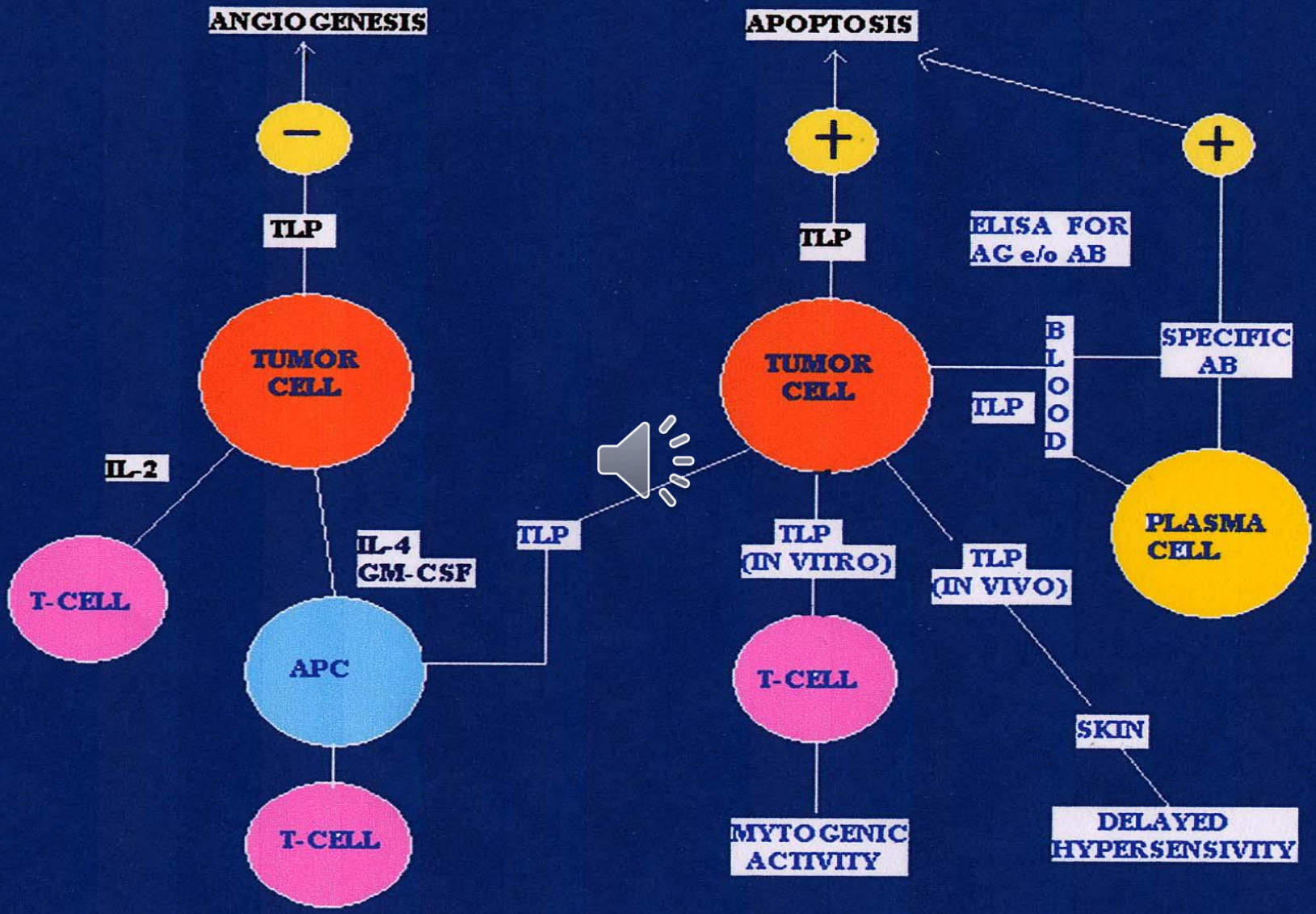
The CD4+ cell releases cytokine molecules that help to activate the CD8+ cells, prompting them to attack other cells with the same antigen

Therapeutic Vaccine Strategies (C)

The dendritic cells of a cancer patient are removed and loaded with antigens from the tumor.

The dendritic cells grow outside the body and then are reinjected, triggering a powerful response by the T cells

2010 – The FDA approves the first therapeutic cancer vaccine for advanced prostate cancer (Provenge).



FOUND AND POTENTIAL USES OF TLP

TLP AS A TUMOR – ASSOCIATED ANTIGEN

- 55 KD PROTEIN OVEREXPRESSED IN LUNG TUMORS AND OTHER EPITHELIAL ADENOCARCINOMAS.

- IMMUNOGENIC IN HUMAN AS EVIDENCED BY SERUM ANTIBODIES

Plasmid name: pGEM -T Easy TLP fragment
Plasmid size: 3.3 kb
Constructed by: Luigi Bagella
Construction date: May '99
Comments: vector:pGEM -T Easy 3.0 Kb (Promega)
insert: RT-PCR from A549 cell line.
The fragment (300bp) could be cleavaged with EcoRI

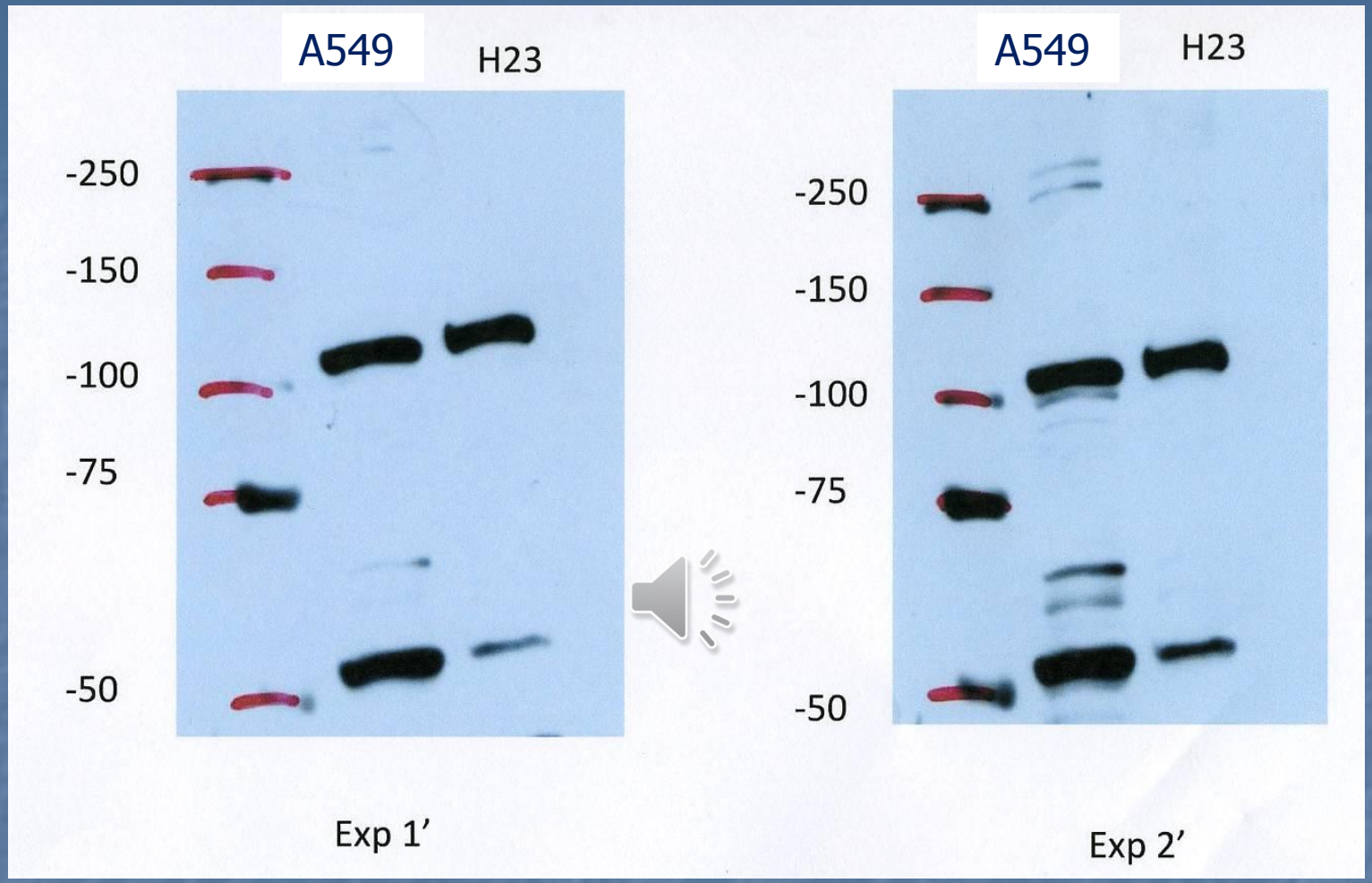
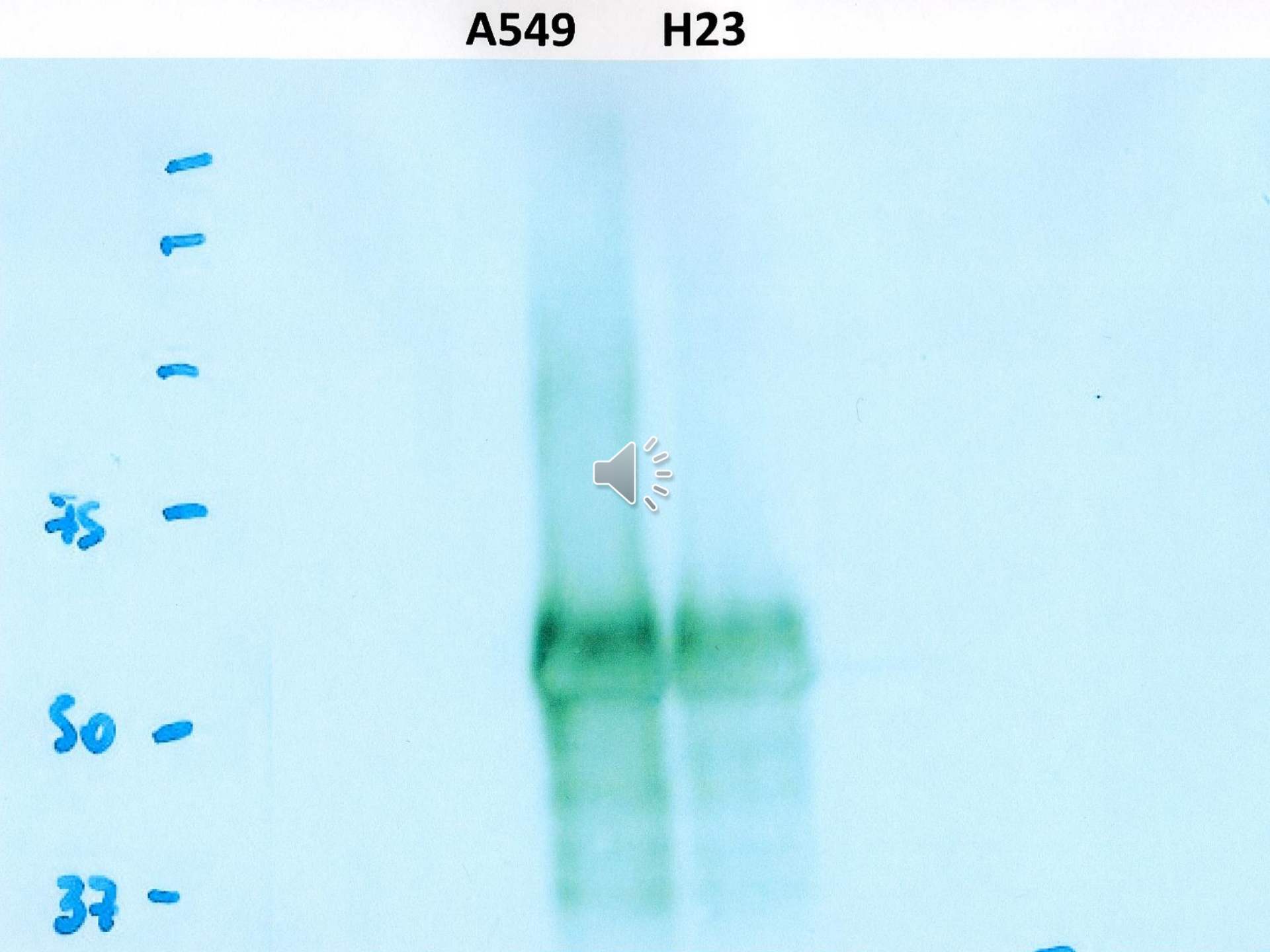


Fig. 1

Western Blot on A549 and H23 Cell Lines.

Two Exposures at Different Times of the Same Experiment


A549
H23
75
50
37

ONCOLOGY
UBP 0011 DIAGNOSTIC

TISSUE MICROARRAY PROFILE (a)

NSCLC STAGE I TISSUES	POSITIVITY (%)	NEGATIVITY (%)
400	56.3 (225/400)	43.7 (175/400)

NORMAL LUNG TISSUES	POSITIVITY (%)	NEGATIVITY (%)
400	0 (0/400)	100 (400/400)

(a) Carried out by William C. Hyun, Ph.D., at the University of California San Francisco, Cancer Center, Laboratory Cell Analysis.

Fugure 1. Western blot with the antiserum anti-TLP and pre-serum in A549 cell.

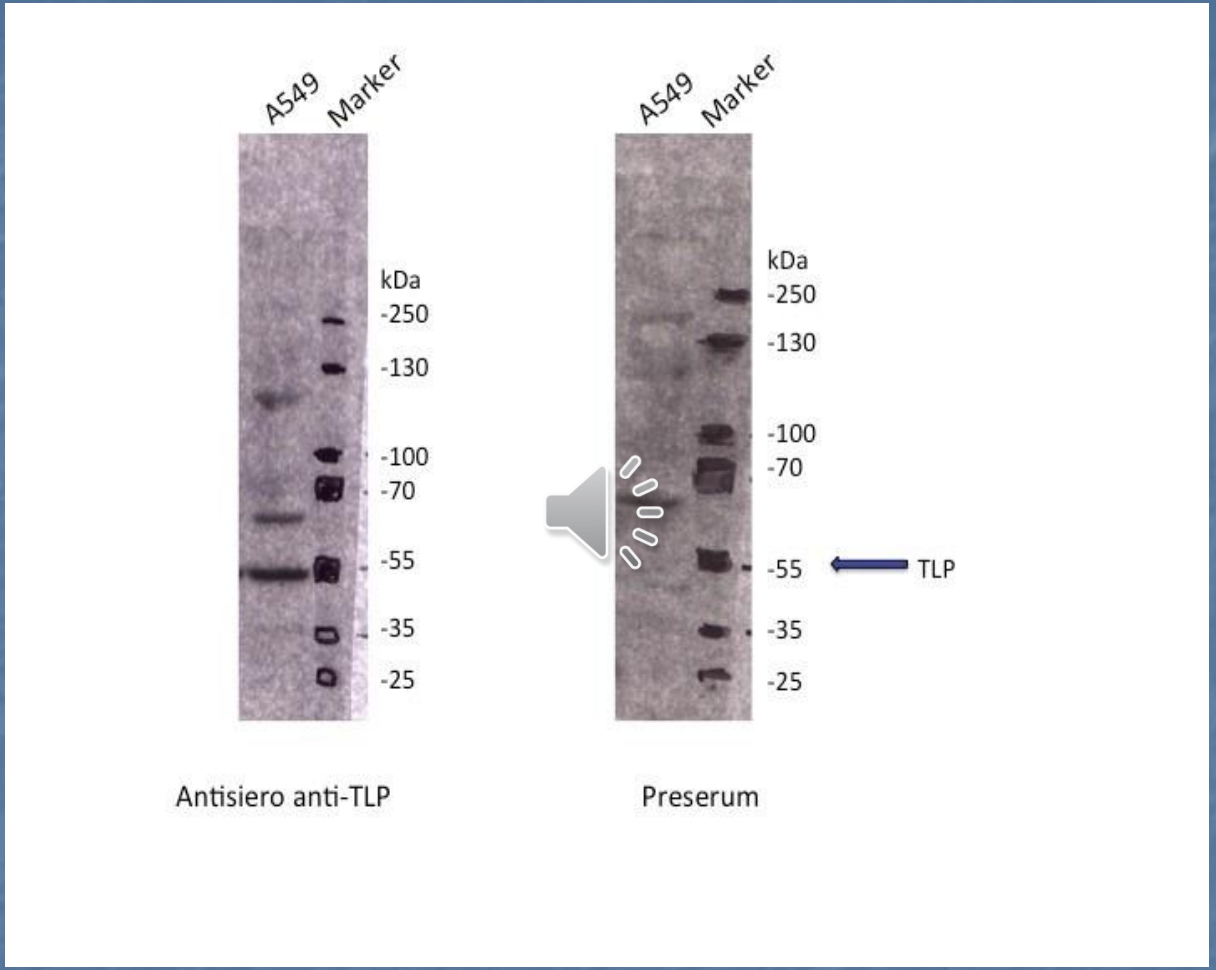


Figura 2. Western blot for PCA assay in A549 cell line. The antiserum was pre-incubated with the peptide RTNKEASI and then hybridized with A549 cell line (panel A). The antiserum was pre-incubated with water and then hybridized with A549 cell line (panel B).

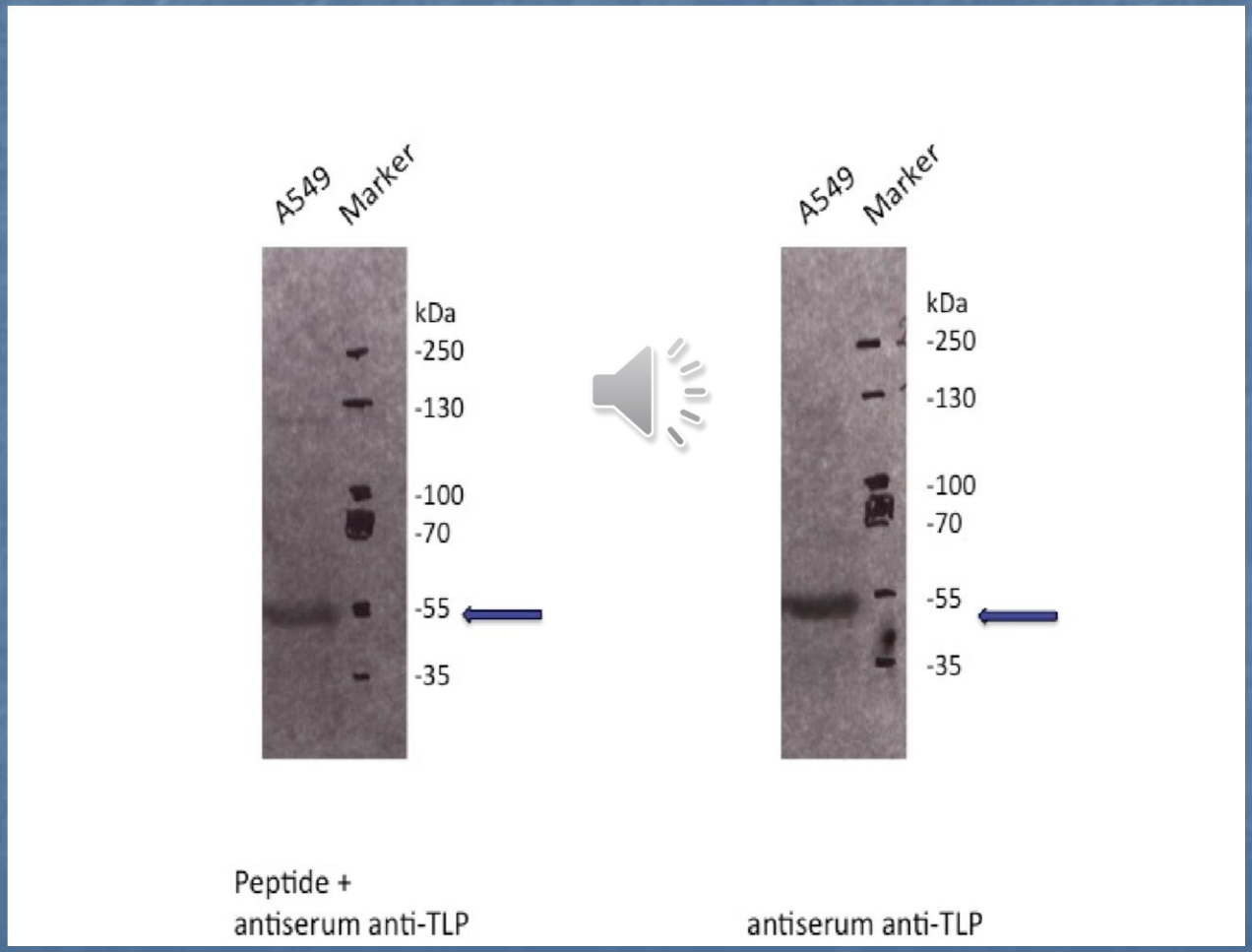


Figure 3. Western blot for the antiserum anti-TLP in A549 cell line, A549 cell culture supernatant (SPN) and MRC 5 human fibroblasts.

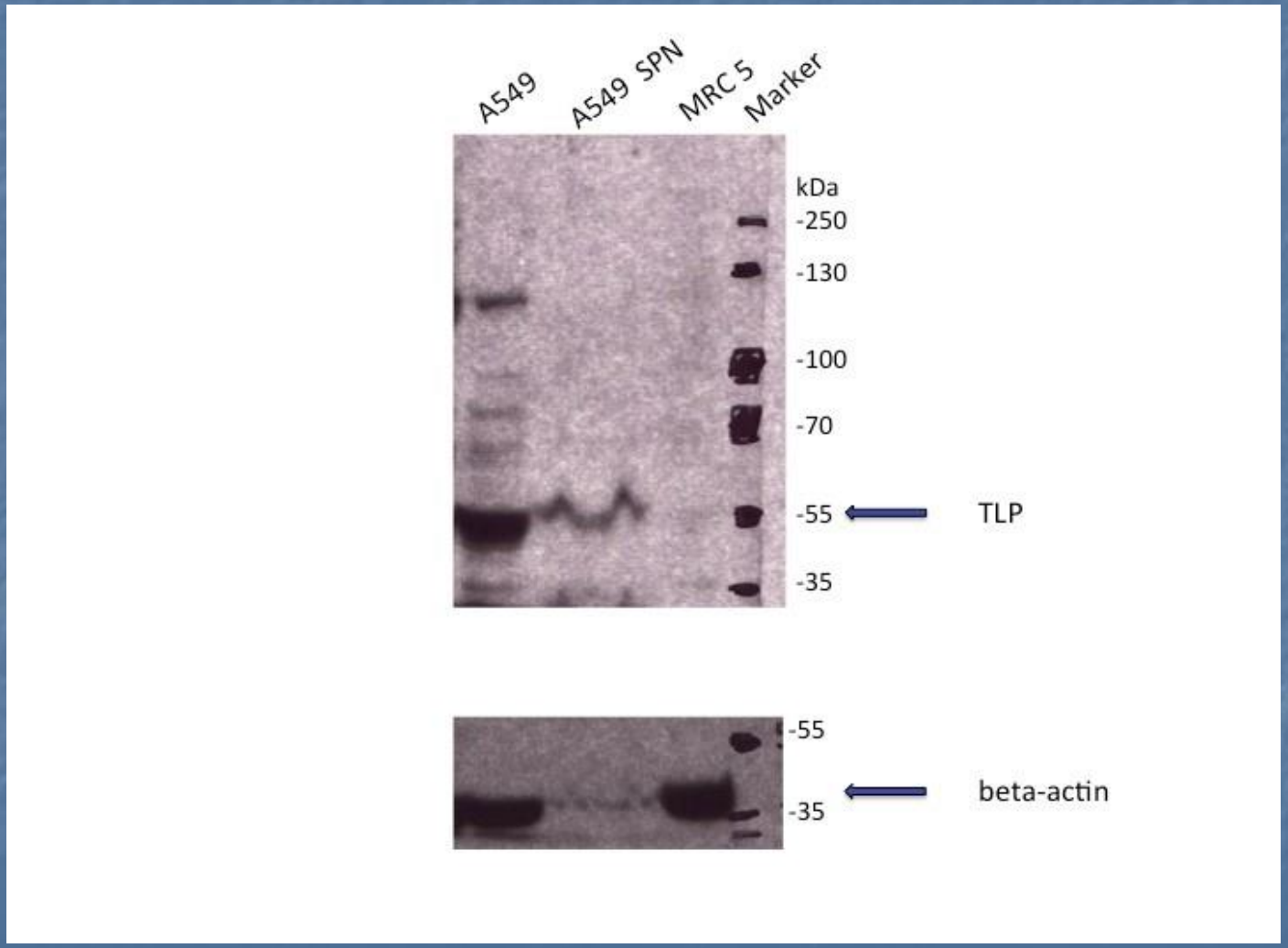


Figure 4. Western blot analysis for the antiserum anti-TLP in Burkitt lymphoma (CA46), leukemia (HL60), breast cancer (MCF7), cervical carcinoma (Hela) and prostate cancer (PC3).

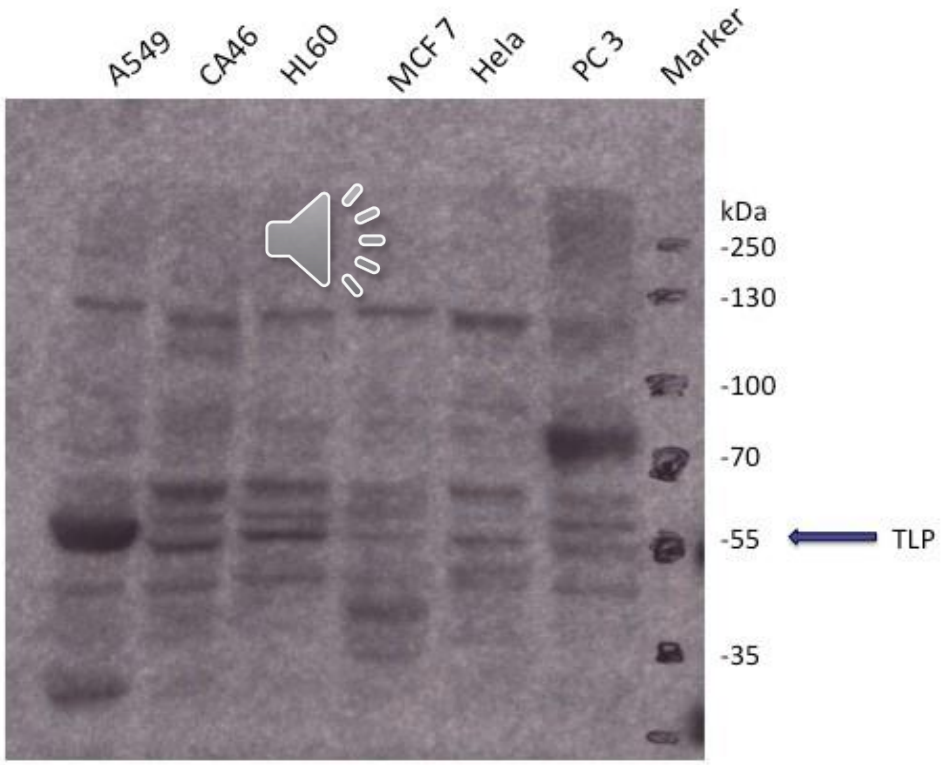
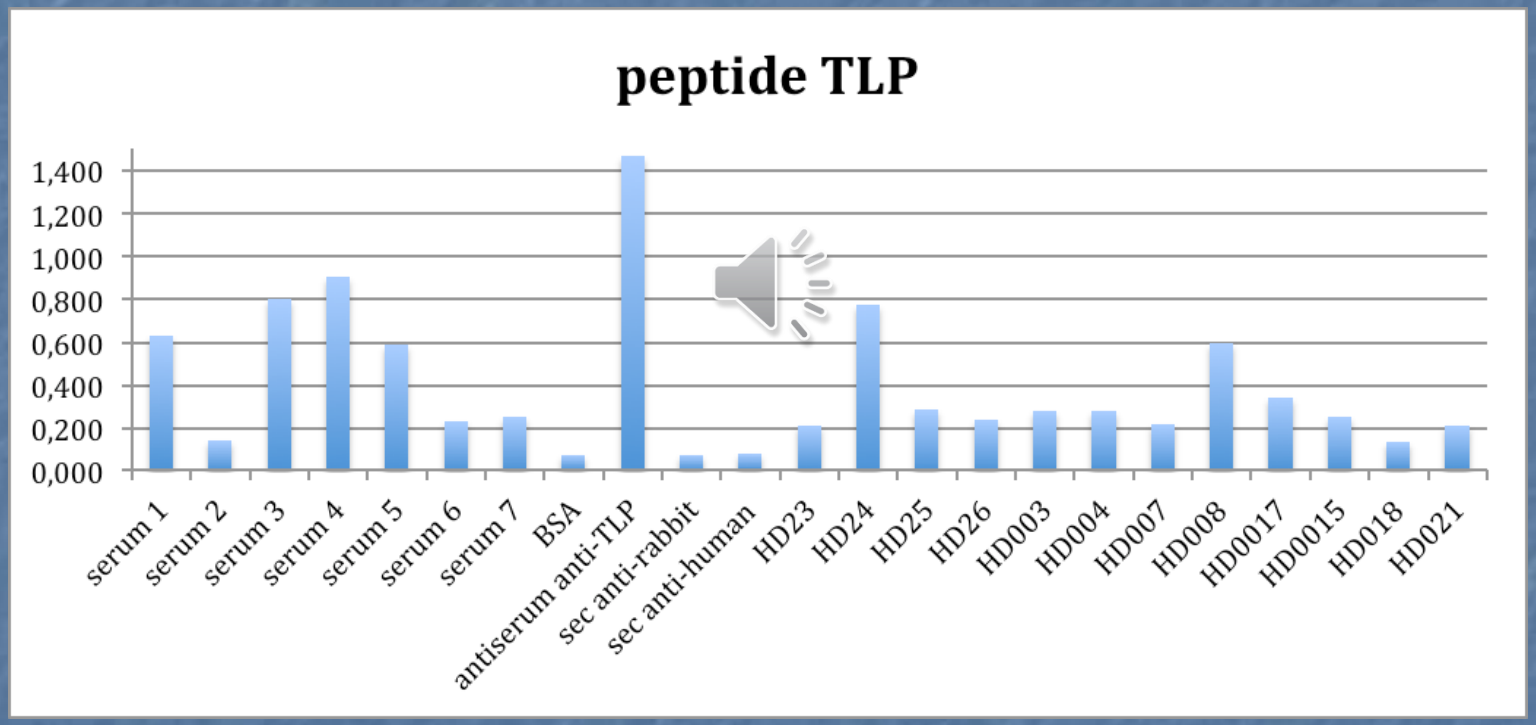
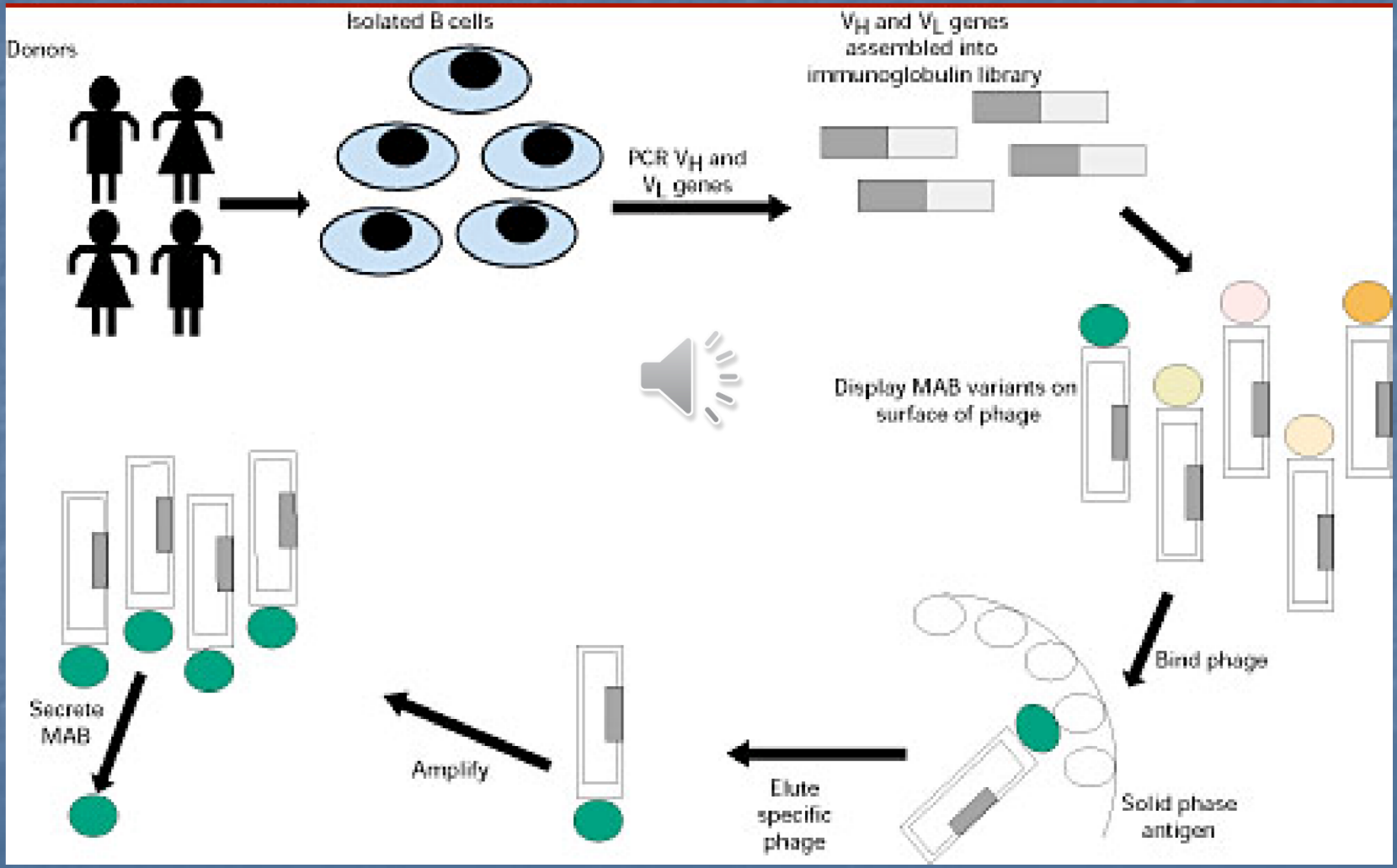


Figure 5. ELISA test in lung cancer patients sera. The peptide RTNKEASI was immobilized into the well and then the reactivity of IgG from lung cancer sera samples (serum1-7) and healthy donors (HD) were determined.



b) Construction of Ab libraries and selection against TLP peptide



This protein band was identified as aldehyde dehydrogenase isoform 1A1 through mass spectrometry, revealing the molecular nature of at least one component of the previously described TLP complex.

a) TLP can be measured in the blood and tumor tissue of patients affected with cancer and therefore it can be used as a diagnostic tool also to monitor the patients response to theraphy.

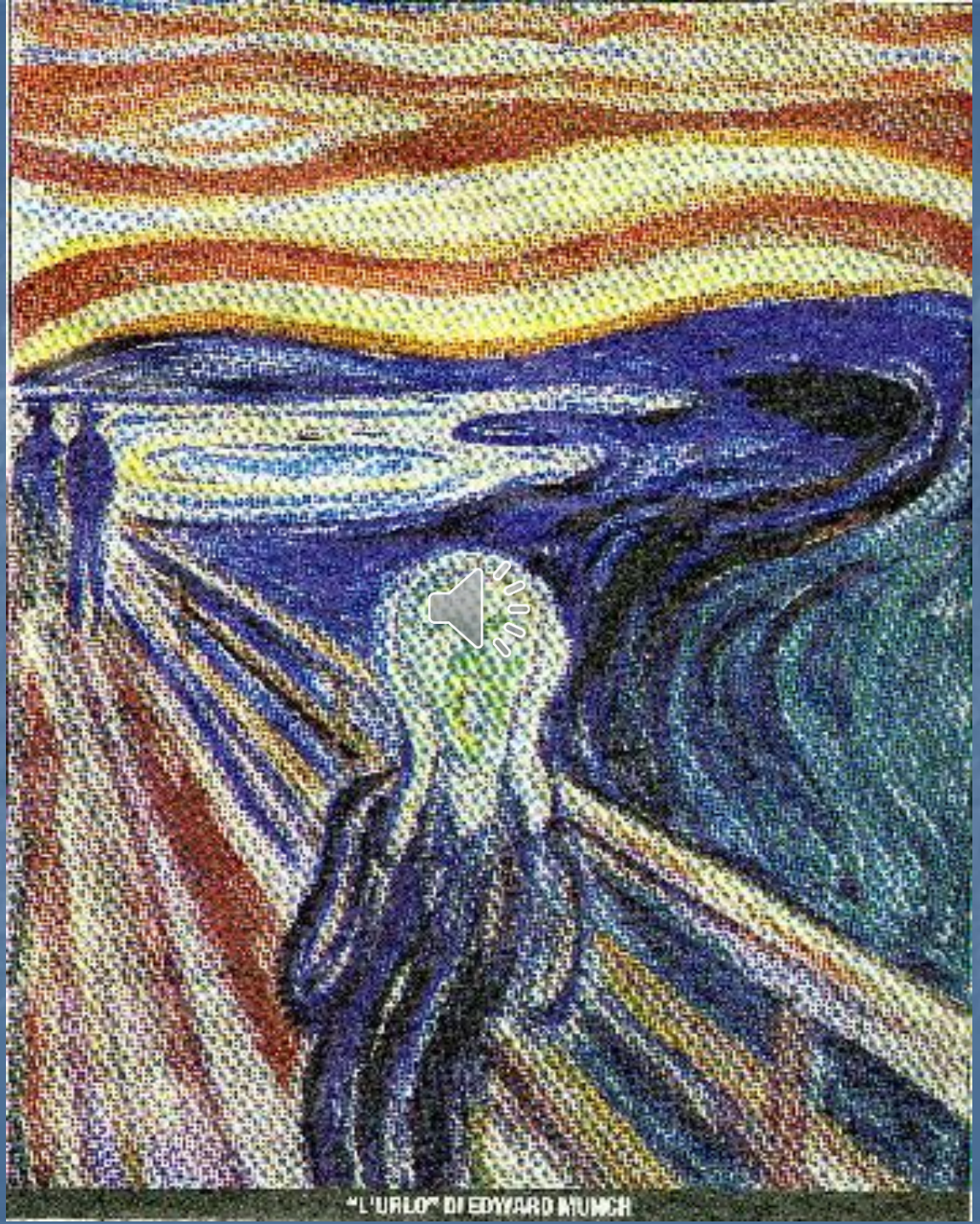
b) TLP can be used to stimulate the cells of the immune system for the treatment of lung cancer patients (or colon cancer patients) in order to prepare a defense against tumor cells that synthesize this protein.

c) TLP can be used to develop a vaccine for the treatment of patients with lung or colon cancer and to prevent neoplastic disease in subjects at risk of developing cancer, through stimulation of the immune system by preparing an attack against cells that express this protein

Immunotherapy Remains Top Cancer Advance, According To ASCO Report.

MedPage Today (2/1, Bankhead, 97K) says, "The transformative effect of immunotherapy on cancer remained the top cancer advance for the second consecutive year, according to the American Society of Clinical Oncology (ASCO)." ASCO officials released the society's 12th annual report, Clinical Cancer Advances 2017, "today during a meeting on Capitol Hill, where they made a pitch for continued – or increased – federal support for cancer research." In addition to immunotherapy, "ASCO singled out three other areas of advancement," which include precision medicine, liquid biopsies, and "physician-patient tools."

The ASCO Post (2/1) adds that "although Congress recently approved funding increases for the National Institutes of Health (NIH) and National Cancer Institute (NCI) for 2017, annual increases that keep pace with inflation are critical to build on the promising research results highlighted in the report."


"L'URLO" DI EDVARD MUNCH